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(57) Abstract

The present invention relates to the discovery of novel protein–protein interactions that are involved in mammalian physiological pathways, including physiological disorders or diseases. Examples of physiological disorders and diseases include non–insulin dependent diabetes mellitus (NIDDM), neurodegenerative disorders, such as Alzheimer's Disease (AD), and the like. Thus, the present invention is directed to complexes of these proteins and/or their fragments, antibodies to the complexes, diagnosis of physiological generative disorders (including diagnosis of a predisposition to and diagnosis of the existence of the disorder), drug screening for agents which modulate the interaction of proteins described herein, and identification of additional proteins in the pathway common to the proteins described herein.

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TITLE OF THE INVENTION

PROTEIN-PROTEIN INTERACTIONS

BACKGROUND OF THE INVENTION

The present invention relates to the discovery of novel protein-protein interactions that are involved in mammalian physiological pathways, including physiological disorders or diseases. Examples of physiological disorders and diseases include non-insulin dependent diabetes mellitus (NIDDM), neurodegenerative disorders, such as Alzheimer's Disease (AD), and the like. Thus, the present invention is directed to complexes of these proteins and/or their fragments, antibodies to the complexes, diagnosis of physiological generative disorders (including diagnosis of a predisposition to and diagnosis of the existence of the disorder), drug screening for agents which modulate the interaction of proteins described herein, and identification of additional proteins in the pathway common to the proteins described herein.

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated herein by reference, and for convenience, are referenced by author and date in the following text and respectively grouped in the appended List of References.

Many processes in biology, including transcription, translation and metabolic or signal transduction pathways, are mediated by non-covalently associated protein complexes. The formation of protein-protein complexes or protein-DNA complexes produce the most efficient chemical machinery. Much of modern biological research is concerned with identifying proteins involved in cellular processes, determining their functions, and how, when and where they interact with other proteins involved in specific pathways. Further, with rapid advances in genome sequencing, there is a need to define protein linkage maps, i.e., detailed inventories of protein interactions that make up functional assemblies of proteins or protein complexes or that make up physiological pathways.

Recent advances in human genomics research has led to rapid progress in the identification of novel genes. In applications to biological and pharmaceutical research, there is a need to determine functions of gene products. A first step in defining the function of a novel gene is to determine its interactions with other gene products in appropriate context. That is, since proteins make specific interactions with other proteins or other biopolymers as part of functional assemblies or physiological pathways, an appropriate way to examine function of a gene is to determine its physical relationship with other genes. Several systems exist for identifying protein interactions and hence relationships between genes.

There continues to be a need in the art for the discovery of additional protein-protein interactions involved in mammalian physiological pathways. There continues to be a need in the art also to identify the protein-protein interactions that are involved in mammalian physiological disorders and diseases, and to thus identify drug targets.

SUMMARY OF THE INVENTION

The present invention relates to the discovery of protein-protein interactions that are involved in mammalian physiological pathways, including physiological disorders or diseases, and to the use of this discovery. The identification of the interacting proteins described herein provide new targets for the identification of useful pharmaceuticals, new targets for diagnostic tools in the identification of individuals at risk, sequences for production of transformed cell lines, cellular models and animal models, and new bases for therapeutic intervention in such physiological pathways

Thus, one aspect of the present invention is protein complexes. The protein complexes are a complex of (a) two interacting proteins, (b) a first interacting protein and a fragment of a second interacting protein, (c) a fragment of a first interacting protein and a second interacting protein, or (d) a fragment of a first interacting protein and a fragment of a second interacting protein. The fragments of the interacting proteins include those parts of the proteins, which interact to form a complex. This aspect of the invention includes the detection of protein interactions and the production of proteins by recombinant techniques. The latter embodiment also includes cloned sequences, vectors, transfected or transformed host cells and transgenic animals.

A second aspect of the present invention is an antibody that is immunoreactive with the above complex. The antibody may be a polyclonal antibody or a monoclonal antibody. While the antibody is immunoreactive with the complex, it is not immunoreactive with the component parts

of the complex. That is, the antibody is not immunoreactive with a first interactive protein, a fragment of a first interacting protein, a second interacting protein or a fragment of a second interacting protein. Such antibodies can be used to detect the presence or absence of the protein complexes.

A third aspect of the present invention is a method for diagnosing a predisposition for physiological disorders or diseases in a human or other animal. The diagnosis of such disorders includes a diagnosis of a predisposition to the disorders and a diagnosis for the existence of the disorders. In accordance with this method, the ability of a first interacting protein or fragment thereof to form a complex with a second interacting protein or a fragment thereof is assayed, or the genes encoding interacting proteins are screened for mutations in interacting portions of the protein molecules. The inability of a first interacting protein or fragment thereof to form a complex, or the presence of mutations in a gene within the interacting domain, is indicative of a predisposition to, or existence of a disorder. In accordance with one embodiment of the invention, the ability to form a complex is assayed in a two-hybrid assay. In a first aspect of this embodiment, the ability to form a complex is assayed by a yeast two-hybrid assay. In a second aspect, the ability to form a complex is assayed by a mammalian two-hybrid assay. In a second embodiment, the ability to form a complex is assayed by measuring *in vitro* a complex formed by combining said first protein and said second protein. In one aspect the proteins are isolated from a human or other animal. In a third embodiment, the ability to form a complex is assayed by measuring the binding of an antibody, which is specific for the complex. In a fourth embodiment, the ability to form a complex is assayed by measuring the binding of an antibody that is specific for the complex with a tissue extract from a human or other animal. In a fifth embodiment, coding sequences of the interacting proteins described herein are screened for mutations.

A fourth aspect of the present invention is a method for screening for drug candidates which are capable of modulating the interaction of a first interacting protein and a second interacting protein. In this method, the amount of the complex formed in the presence of a drug is compared with the amount of the complex formed in the absence of the drug. If the amount of complex formed in the presence of the drug is greater than or less than the amount of complex formed in the absence of the drug, the drug is a candidate for modulating the interaction of the first and second interacting proteins. The drug promotes the interaction if the complex formed in the presence of the drug is greater and inhibits (or disrupts) the interaction if the complex formed in the presence of the

drug is less. The drug may affect the interaction directly, i.e., by modulating the binding of the two proteins, or indirectly, e.g., by modulating the expression of one or both of the proteins.

A fifth aspect of the present invention is a model for such physiological pathways, disorders or diseases. The model may be a cellular model or an animal model, as further described herein. In accordance with one embodiment of the invention, an animal model is prepared by creating transgenic or "knock-out" animals. The knock-out may be a total knock-out, i.e., the desired gene is deleted, or a conditional knock-out, i.e., the gene is active until it is knocked out at a determined time. In a second embodiment, a cell line is derived from such animals for use as a model. In a third embodiment, an animal model is prepared in which the biological activity of a protein complex of the present invention has been altered. In one aspect, the biological activity is altered by disrupting the formation of the protein complex, such as by the binding of an antibody or small molecule to one of the proteins which prevents the formation of the protein complex. In a second aspect, the biological activity of a protein complex is altered by disrupting the action of the complex, such as by the binding of an antibody or small molecule to the protein complex which interferes with the action of the protein complex as described herein. In a fourth embodiment, a cell model is prepared by altering the genome of the cells in a cell line. In one aspect, the genome of the cells is modified to produce at least one protein complex described herein. In a second aspect, the genome of the cells is modified to eliminate at least one protein of the protein complexes described herein.

A sixth aspect of the present invention are nucleic acids coding for novel proteins discovered in accordance with the present invention.

A seventh aspect of the present invention is a method of screening for drug candidates useful for treating a physiological disorder. In this embodiment, drugs are screened on the basis of the association of a protein with a particular physiological disorder. This association is established in accordance with the present invention by identifying a relationship of the protein with a particular physiological disorder. The drugs are screened by comparing the activity of the protein in the presence and absence of the drug. If a difference in activity is found, then the drug is a drug candidate for the physiological disorder. The activity of the protein can be assayed *in vitro* or *in vivo* using conventional techniques, including transgenic animals and cell lines of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is the discovery of novel interactions between proteins described herein. The genes coding for some of these proteins may have been cloned previously, but their potential interaction in a physiological pathway or with a particular protein was unknown. Alternatively, the genes coding for some of these proteins have not been cloned previously and represent novel genes. These proteins are identified using the yeast two-hybrid method and searching a human total brain library, as more fully described below.

According to the present invention, new protein-protein interactions have been discovered. The discovery of these interactions has identified several protein complexes for each protein-protein interaction. The protein complexes for these interactions are set forth below in Tables 1-73, which also identify the new protein-protein interactions of the present invention.

TABLE 1

Protein Complexes of Glut4/CARP Interaction

Glucose Transporter 4 (Glut4) and Clone C-193 (CARP)
A fragment of Glut4 and CARP
Glut4 and a fragment of CARP
A fragment of Glut4 and a fragment of CARP

TABLE 2

Protein Complexes of Glut1/DRAL(FHL2) Interaction

Glucose Transporter 1 (Glut1) and DRAL(FHL2)
A fragment of Glut1 and DRAL(FHL2)
Glut1 and a fragment of DRAL(FHL2)
A fragment of Glut1 and a fragment of DRAL(FHL2)

TABLE 3

Protein Complexes of Glut1/Myosin Heavy Chain Interaction

Glucose Transporter 1 (Glut1) and myosin heavy chain
A fragment of Glut1 and myosin heavy chain
Glut1 and a fragment of myosin heavy chain
A fragment of Glut1 and a fragment of myosin heavy chain

TABLE 4

Protein Complexes of Glut1/HSS Interaction

Glucose Transporter 1 (Glut1) and human sperm surface protein (HSS)
A fragment of Glut1 and HSS
Glut1 and a fragment of HSS
A fragment of Glut1 and a fragment of HSS

TABLE 5

Protein Complexes of OGTase/Myosin Heavy Chain Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and myosin heavy chain
A fragment of OGTase and myosin heavy chain
OGTase and a fragment of myosin heavy chain
A fragment of OGTase and a fragment of myosin heavy chain

TABLE 6

Protein Complexes of IRAP/14-3-3 Beta Interaction

Insulin-Regulated Membrane-Spanning Aminopeptidase (IRAP) and 14-3-3 beta
A fragment of IRAP and 14-3-3 beta
IRAP and a fragment of 14-3-3 beta
A fragment of IRAP and a fragment of 14-3-3 beta

TABLE 7

Protein Complexes of IRAP/HSS Interaction

Insulin-Regulated Membrane-Spanning Aminopeptidase (IRAP) and human sperm surface protein (HSS)
A fragment of IRAP and HSS
IRAP and a fragment of HSS
A fragment of IRAP and a fragment of HSS

TABLE 8

Protein Complexes of PI-3K110/Complement Protein C4 Interaction

PI-3 Kinase p110 subunit (PI-3K110) and Complement Protein C4
A fragment of PI-3K110 and Complement Protein C4
PI-3K110 and a fragment of Complement Protein C4
A fragment of PI-3K110 and a fragment of Complement Protein C4

TABLE 9

Protein Complexes of PI-3K110/Tenascin XB Interaction

PI-3 Kinase p110 subunit (PI-3K110) and Tenascin XB
A fragment of PI-3K110 and Tenascin XB
PI-3K110 and a fragment of Tenascin XB
A fragment of PI-3K110 and a fragment of Tenascin XB

TABLE 10

Protein Complexes of PI-3K110/GAA Interaction

PI-3 Kinase p110 subunit (PI-3K110) and Alpha Acid Glucosidase (GAA)
A fragment of PI-3K110 and GAA
PI-3K110 and a fragment of GAA
A fragment of PI-3K110 and a fragment of GAA

TABLE 11

Protein Complexes of MM-1/C-Nap1 Interaction

C-myc Binding Protein (MM-1) and C-Nap1
A fragment of MM-1 and C-Nap1
MM-1 and a fragment of C-Nap1
A fragment of MM-1 and a fragment of C-Nap1

TABLE 12

Protein Complexes of MM-1/Beta Spectrin Interaction

C-myc Binding Protein (MM-1) and Beta Spectrin
A fragment of MM-1 and Beta Spectrin
MM-1 and a fragment of Beta Spectrin
A fragment of MM-1 and a fragment of Beta Spectrin

TABLE 13

Protein Complexes of MM-1/KIAA0477 Interaction

C-myc Binding Protein (MM-1) and KIAA0477
A fragment of MM-1 and KIAA0477
MM-1 and a fragment of KIAA0477
A fragment of MM-1 and a fragment of KIAA0477

TABLE 14

Protein Complexes of Dynamin/CALM Interaction

Dynamin and Calthrin Assembly Protein (CALM)
A fragment of Dynamin and CALM
Dynamin and a fragment of CALM
A fragment of Dynamin and a fragment of CALM

TABLE 15

Protein Complexes of Dynamin/Psme3 Interaction

Dynamin and Proteosome Activator Subunit Psme3 (Psme3)
A fragment of Dynamin and Psme3
Dynamin and a fragment of Psme3
A fragment of Dynamin and a fragment of Psme3

TABLE 16

Protein Complexes of Naf1b/I-TRAF Interaction

Nef-Associated Factor 1 beta (Naf1b) and I-TRAF
A fragment of Naf1b and I-TRAF
Naf1b and a fragment of I-TRAF
A fragment of Naf1b and a fragment of I-TRAF

TABLE 17

Protein Complexes of Akt1/NuMA1 Interaction

Akt kinase 1 (Akt1) and NuMA1
A fragment of Akt1 and NuMA1
Akt1 and a fragment of NuMA1
A fragment of Akt1 and a fragment of NuMA1

TABLE 18

Protein Complexes of Akt2/NuMA1 Interaction

Akt kinase 2 (Akt2) and NuMA1
A fragment of Akt2 and NuMA1
Akt2 and a fragment of NuMA1
A fragment of Akt2 and a fragment of NuMA1

TABLE 19

Protein Complexes of Akt2/BAP31 Interaction

Akt kinase 2 (Akt2) and BAP31
A fragment of Akt2 and BAP31
Akt2 and a fragment of BAP31
A fragment of Akt2 and a fragment of BAP31

TABLE 20

Protein Complexes of Akt2/Beta Adaptin Interaction

Akt kinase 2 (Akt2) and beta adaptin
A fragment of Akt2 and beta adaptin
Akt2 and a fragment of beta adaptin
A fragment of Akt2 and a fragment of beta adaptin

TABLE 21

Protein Complexes of OGTase/Desmin Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and desmin
A fragment of OGTase and desmin
OGTase and a fragment of desmin
A fragment of OGTase and a fragment of desmin

TABLE 22

Protein Complexes of OGTase/Alpha-karyopherin Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and alpha-karyopherin
A fragment of OGTase and alpha-karyopherin
OGTase and a fragment of alpha-karyopherin
A fragment of OGTase and a fragment of alpha-karyopherin

TABLE 23

Protein Complexes of OGTase/Glutaminyl tRNA Synthetase Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and glutaminyl tRNA synthetase
A fragment of OGTase and glutaminyl tRNA synthetase
OGTase and a fragment of glutaminyl tRNA synthetase
A fragment of OGTase and a fragment of glutaminyl tRNA synthetase

TABLE 24

Protein Complexes of OGTase/Clone 25100 Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and clone 25100
A fragment of OGTase and clone 25100
OGTase and a fragment of clone 25100
A fragment of OGTase and a fragment of clone 25100

TABLE 25

Protein Complexes of PTP1b/VAP-A Interaction

PTP1b and VAMP-associated protein A (VAP-A)
A fragment of PTP1b and VAP-A
PTP1b and a fragment of VAP-A
A fragment of PTP1b and a fragment of VAP-A

TABLE 26

Protein Complexes of Rab4/Alpha-catenin-like Protein Interaction

Rab4 and alpha-cantein-like protein
A fragment of Rab4 and alpha-cantein-like protein
Rab4 and a fragment of alpha-cantein-like protein
A fragment of Rab4 and a fragment of alpha-cantein-like protein

TABLE 27

Protein Complexes of Rab4/Rab2 Interaction

Rab4 and Rab2
A fragment of Rab4 and Rab2
Rab4 and a fragment of Rab2
A fragment of Rab4 and a fragment of Rab2

TABLE 28

Protein Complexes of Glut4/PN7065 Interaction

Glucose Transporter 4 (Glut4) and Novel Protein Fragment PN7065 (PN7065)
A fragment of Glut4 and PN7065
Glut4 and a fragment of PN7065
A fragment of Glut4 and a fragment of PN7065

TABLE 29

Protein Complexes of Glut4/PN7386 Interaction

Glucose Transporter 4 (Glut4) and Novel Protein Fragment PN7386 (PN7386)
A fragment of Glut4 and PN7386
Glut4 and a fragment of PN7386
A fragment of Glut4 and a fragment of PN7386

TABLE 30

Protein Complexes of OGTase/PN6931 Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and Novel Protein Fragment PN6931 (PN6931)
A fragment of OGTase and PN6931
OGTase and a fragment of PN6931
A fragment of OGTase and a fragment of PN6931

TABLE 31

Protein Complexes of Naf1b/PN7582 Interaction

Nef-Associated Factor 1 beta (Naf1b) and Novel Protein Fragment PN7582 (PN7582)
A fragment of Naf1b and PN7582
Naf1b and a fragment of PN7582
A fragment of Naf1b and a fragment of PN7582

TABLE 32

Protein Complexes of OGTase/Talin Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and Talin
A fragment of OGTase and Talin
OGTase and a fragment of Talin
A fragment of OGTase and a fragment of Talin

TABLE 33

Protein Complexes of OGTase/MOP2 Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and MOP2
A fragment of OGTase and MOP2
OGTase and a fragment of MOP2
A fragment of OGTase and a fragment of MOP2

TABLE 34

Protein Complexes of OGTase/Clone 25100 Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and Clone 25100
A fragment of OGTase and Clone 25100
OGTase and a fragment of Clone 25100
A fragment of OGTase and a fragment of Clone 25100

TABLE 35

Protein Complexes of OGTase/KIAA0443 Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and KIAA0443
A fragment of OGTase and KIAA0443
OGTase and a fragment of KIAA0443
A fragment of OGTase and a fragment of KIAA0443

TABLE 36

Protein Complexes of OGTase/EGR1 Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and EGR1
A fragment of OGTase and EGR1
OGTase and a fragment of EGR1
A fragment of OGTase and a fragment of EGR1

TABLE 37

Protein Complexes of OGTase/Dynamin II Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and Dynamin II
A fragment of OGTase and Dynamin II
OGTase and a fragment of Dynamin II
A fragment of OGTase and a fragment of Dynamin II

TABLE 38

Protein Complexes of OGTase/INT-6 Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and INT-6
A fragment of OGTase and INT-6
OGTase and a fragment of INT-6
A fragment of OGTase and a fragment of INT-6

TABLE 39

Protein Complexes of OGTase/HSPC028 Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and HSPC028
A fragment of OGTase and HSPC028
OGTase and a fragment of HSPC028
A fragment of OGTase and a fragment of HSPC028

TABLE 40

Protein Complexes of OGTase/BAP31 Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and BAP31
A fragment of OGTase and BAP31
OGTase and a fragment of BAP31
A fragment of OGTase and a fragment of BAP31

TABLE 41

Protein Complexes of OGTase/Interferon-Ind Prot Interaction

O-linked N-acetylglucosaminyltransferase (OGTase) and Interferon-Ind Protein
A fragment of OGTase and Interferon-Ind Protein
OGTase and a fragment of Interferon-Ind Protein
A fragment of OGTase and a fragment of Interferon-Ind Protein

TABLE 42

Protein Complexes of Glut4/Beta-Catenin Interaction

Glucose Transporter 4 (Glut4) and Beta-catenin
A fragment of Glut4 and Beta-catenin
Glut4 and a fragment of Beta-catenin
A fragment of Glut4 and a fragment of Beta-catenin

TABLE 43

Protein Complexes of Glut4/Alpha-SNAP Interaction

Glucose Transporter 4 (Glut4) and Alpha-SNAP
A fragment of Glut4 and Alpha-SNAP
Glut4 and a fragment of Alpha-SNAP
A fragment of Glut4 and a fragment of Alpha-SNAP

TABLE 44

Protein Complexes of Glut4/MAPKKK6 Interaction

Glucose Transporter 4 (Glut4) and MAPKKK6
A fragment of Glut4 and MAPKKK6
Glut4 and a fragment of MAPKKK6
A fragment of Glut4 and a fragment of MAPKKK6

TABLE 45

Protein Complexes of Glut4/Tropomyosin 3 Interaction

Glucose Transporter 4 (Glut4) and Tropomyosin 3
A fragment of Glut4 and Tropomyosin 3
Glut4 and a fragment of Tropomyosin 3
A fragment of Glut4 and a fragment of Tropomyosin 3

TABLE 46

Protein Complexes of Glut1/DRAL/FHL2 Interaction

Glucose Transporter 1 (Glut1) and DRAL/FHL2
A fragment of Glut1 and DRAL/FHL2
Glut1 and a fragment of DRAL/FHL2
A fragment of Glut1 and a fragment of DRAL/FHL2

TABLE 47

Protein Complexes of Glut1/MYSA Interaction

Glucose Transporter 1 (Glut1) and cardiac muscle myosin heavy chain (MYSA)
A fragment of Glut1 and MYSA
Glut1 and a fragment of MYSA
A fragment of Glut1 and a fragment of MYSA

TABLE 48

Protein Complexes of IRAP/SLAP-2 Interaction

Insulin-Regulated Membrane-Spanning Aminopeptidase (IRAP) and SLAP-2
A fragment of IRAP and SLAP-2
IRAP and a fragment of SLAP-2
A fragment of IRAP and a fragment of SLAP-2

TABLE 49

Protein Complexes of IRAP/SG2NA Interaction

Insulin-Regulated Membrane-Spanning Aminopeptidase (IRAP) and SG2NA
A fragment of IRAP and SG2NA
IRAP and a fragment of SG2NA
A fragment of IRAP and a fragment of SG2NA

TABLE 50

Protein Complexes of OGTase/14-3-3-epsilon

O-linked N-acetylglucosaminyltransferase (OGTase) and 14-3-3-epsilon
A fragment of OGTase and 14-3-3-epsilon
OGTase and a fragment of 14-3-3-epsilon
A fragment of OGTase and a fragment of 14-3-3-epsilon

TABLE 51

Protein Complexes of PI-3K85/Chromogranin Interaction

PI-3 Kinase p85 subunit (PI-3K85) and Chromogranin
A fragment of PI-3K85 and Chromogranin
PI-3K85 and a fragment of Chromogranin
A fragment of PI-3K85 and a fragment of Chromogranin

TABLE 52

Protein Complexes of PI-3K85/SLP-76 Interaction

PI-3 Kinase p85 subunit (PI-3K85) and SLP-76
A fragment of PI-3K85 and SLP-76
PI-3K85 and a fragment of SLP-76
A fragment of PI-3K85 and a fragment of SLP-76

TABLE 53

Protein Complexes of PI-3K85/14-3-3-zeta Interaction

PI-3 Kinase p85 subunit (PI-3K85) and 14-3-3-zeta
A fragment of PI-3K85 and 14-3-3-zeta
PI-3K85 and a fragment of 14-3-3-zeta
A fragment of PI-3K85 and a fragment of 14-3-3-zeta

TABLE 54

Protein Complexes of PI-3K85/14-3-3-eta Interaction

PI-3 Kinase p85 subunit (PI-3K85) and 14-3-3-eta
A fragment of PI-3K85 and 14-3-3-eta
PI-3K85 and a fragment of 14-3-3-eta
A fragment of PI-3K85 and a fragment of 14-3-3-eta

TABLE 55

Protein Complexes of PI-3K85/TACC2 Interaction

PI-3 Kinase p85 subunit (PI-3K85) and TACC2
A fragment of PI-3K85 and TACC2
PI-3K85 and a fragment of TACC2
A fragment of PI-3K85 and a fragment of TACC2

TABLE 56

Protein Complexes of Glut4/MM-1 Interaction

Glucose Transporter 4 (Glut4) and C-Myc Binding Protein (MM-1)
A fragment of Glut4 and MM-1
Glut4 and a fragment of MM-1
A fragment of Glut4 and a fragment of MM-1

TABLE 57

Protein Complexes of Glut1/KIAA0144 Interaction

Glucose Transporter 1 (Glut1) and KIAA0144 (KIAA)
A fragment of Glut1 and KIAA
Glut1 and a fragment of KIAA
A fragment of Glut1 and a fragment of KIAA

TABLE 58

Protein Complexes of Glut1/Dynamin Interaction

Glucose Transporter 1 (Glut1) and Dynamin
A fragment of Glut1 and Dynamin
Glut1 and a fragment of Dynamin
A fragment of Glut1 and a fragment of Dynamin

TABLE 59

Protein Complexes of Glut1/Clone 25204 Interaction

Glucose Transporter 1 (Glut1) and Clone 25204
A fragment of Glut1 and Clone 25204
Glut1 and a fragment of Clone 25204
A fragment of Glut1 and a fragment of Clone 25204

TABLE 60

Protein Complexes of IRAP/VAP-A Interaction

Insulin-Regulated Membrane-Spanning Aminopeptidase (IRAP; oxytocinase) and VAMP-Associated Protein A (VAP-A)
A fragment of IRAP and VAP-A
IRAP and a fragment of VAP-A
A fragment of IRAP and a fragment of VAP-A

TABLE 61

Protein Complexes of OGTase/Nafla Interaction

O-Linked-N-AcetylglucosaminylTransferase (OGTase) and NEF-Associated Factor 1 Alpha (Nafla)
A fragment of OGTase and Nafla
OGTase and a fragment of Nafla
A fragment of OGTase and a fragment of Nafla

TABLE 62

Protein Complexes of OGTase/Alpha-2-Catenin Interaction

O-Linked-N-AcetylglucosaminylTransferase (OGTase) and Alpha-2-Catenin
A fragment of OGTase and Alpha-2-Catenin
OGTase and a fragment of Alpha-2-Catenin
A fragment of OGTase and a fragment of Alpha-2-Catenin

TABLE 63

Protein Complexes of PI-3K110/TRIP15 Interaction

PI-3 Kinase p110 subunit (PI-3K110) and TRIP15
A fragment of PI-3K110 and TRIP15
PI-3K110 and a fragment of TRIP15
A fragment of PI-3K110 and a fragment of TRIP15

TABLE 64

Protein Complexes of Glut4/14-3-3 Zeta Interaction

Glucose Transporter 4 (Glut4) and 14-3-3 Zeta
A fragment of Glut4 and 14-3-3 Zeta
Glut4 and a fragment of 14-3-3 Zeta
A fragment of Glut4 and a fragment of 14-3-3 Zeta

TABLE 65

Protein Complexes of Glut4/KIAA0282 Interaction

Glucose Transporter 4 (Glut4) and KIAA0282 (an efp-like protein)
A fragment of Glut4 and KIAA0282
Glut4 and a fragment of KIAA0282
A fragment of Glut4 and a fragment of KIAA0282

TABLE 66

Protein Complexes of Glut4/Tankyrase Interaction

Glucose Transporter 4 (Glut4) and Tankyrase
A fragment of Glut4 and Tankyrase
Glut4 and a fragment of Tankyrase
A fragment of Glut4 and a fragment of Tankyrase

TABLE 67

Protein Complexes of IRAP/PTPZ Interaction

Insulin-Regulated Membrane-Spanning Aminopeptidase (IRAP) and protein tyrosine phosphatase zeta (PTPZ)
A fragment of IRAP and PTPZ
IRAP and a fragment of PTPZ
A fragment of IRAP and a fragment of PTPZ

TABLE 68

Protein Complexes of IRAP/βSpectrin Interaction

Insulin-Regulated Membrane-Spanning Aminopeptidase (IRAP) and β-spectrin
A fragment of IRAP and β-spectrin
IRAP and a fragment of β-spectrin
A fragment of IRAP and a fragment of β-spectrin

TABLE 69

Protein Complexes of IRAP/PI-3K85 Interaction

Insulin-Regulated Membrane-Spanning Aminopeptidase (IRAP) and PI-3 Kinase p85 subunit (PI-3K85)
A fragment of IRAP and PI-3K85
IRAP and a fragment of PI-3K85
A fragment of IRAP and a fragment of PI-3K85

TABLE 70

Protein Complexes of PP5/HSP89 Interaction

Protein Phosphatase 5 (PP5) and Heat Shock Protein 89 (HSP89)
A fragment of PP5 and HSP89
PP5 and a fragment of HSP89
A fragment of PP5 and a fragment of HSP89

TABLE 71

Protein Complexes of PP5/Tankyrase Interaction

Protein Phosphatase 5 (PP5) and Tankyrase
A fragment of PP5 and Tankyrase
PP5 and a fragment of Tankyrase
A fragment of PP5 and a fragment of Tankyrase

TABLE 72

Protein Complexes of PI-3K85/Tankyrase Interaction

PI-3 Kinase p85 subunit (PI-3K85) and Tankyrase
A fragment of PI-3K85 and Tankyrase
PI-3K85 and a fragment of Tankyrase
A fragment of PI-3K85 and a fragment of Tankyrase

TABLE 73

Protein Complexes of PI-3K110/APP Interaction

PI-3 Kinase p110 subunit (PI-3K110) and Amyloid Precursor Protein (APP)
A fragment of PI-3K110 and APP
PI-3K110 and a fragment of APP
A fragment of PI-3K110 and a fragment of APP

The proteins newly associated with a particular physiological pathway described herein are used to screen for drug candidates useful for treating a physiological disorder. These proteins are set forth in Table 74.

TABLE 74

Proteins for Drug Screening and Physiological Disorder

PI-3K110	Alzheimer's Disease
PI-3K110	Diabetes
TRIP15	Alzheimer's Disease
TRIP15	Diabetes
All Others	Diabetes

The involvement of above interactions in particular pathways is as follows.

One of the key questions which must be answered in order to understand and treat non-insulin dependent diabetes mellitus (NIDDM) is how glucose uptake is regulated in the cell. It is known that in adipose and muscle tissues there exists an insulin-regulated membrane-spanning glucose transporter called Glut4 that shuttles between the interior of the cell to the plasma membrane by means of vesicle-mediated endocytosis and exocytosis (Garvey et al., 1998; Haruta et al.,1995; Pessin et al., 1999). Fat and muscle cells from diabetic patients appear to be defective in this type of regulated glucose transport (Zierath et al., 1998). The mechanisms by which this transport to the plasma membrane and return back to the interior of the cell are regulated are not well understood. Toward this end, there has been much interest in analyzing the Glut4 protein and the additional factors that participate in glucose uptake in fat and muscle cells. As described below, the yeast two-hybrid assay has been employed as a means of identifying proteins that interact with Glut4 as well as other molecules that have been implicated in the insulin-dependent transport of glucose. Thus, by understanding the regulation of glucose transport in normal cells, medical interventions can be discovered that would serve to increase sugar uptake in the cells of diabetic patients.

One approach to understanding how glucose transport is achieved has been to use the Glut4 molecule as a molecular bait in the search for other molecules which can interact with it. In this way, it might be possible to find ways to influence the function of Glut4 and perhaps find ways to cause it to go to the plasma membrane and subsequently remove glucose from the outside of the cell and bring it into the interior. Using the yeast two-hybrid assay, we have been able to demonstrate

that clone C-193 (known as CARP in other systems) can bind to Glut4. CARP is a cytokine-inducible gene that reportedly acts as a negative regulator of cardiac-specific genes (Jeyaseelan et al., 1997). This interaction may serve as a tie between heart disease and diabetes.

Using the yeast two-hybrid assay, we have also been able to detect the interaction of Glut4 with two novel proteins that have been named PN7065 and PN7386. PN7065 bears a striking similarity to a rat salt-induced protein kinase (GenBank accession AB020480). Experiments in rats have shown that this salt-induced kinase may play an important role in the regulation of adrenocortical functions in response to high plasma salt and ACTH stimulation (Wang et al., 1999). It is possible that Glut4 may act as a substrate for the kinase. PN7386 is identical to a human chromosome 20 clone called 850H21 (GenBank accession AL031680) that is uncharacterized in the literature. It is possible that the protein product of this clone may participate in protein trafficking or in the signal transduction mechanism that regulates this process.

Using the yeast two-hybrid assay, we have been able to detect four more proteins, beta-catenin, alpha-SNAP, tropomyosin 3 and MAPKKK6, which can bind to Glut4. Beta-catenin is a protein containing so-called Armadillo repeats that is involved in two important cellular processes: signal transduction via the Wingless pathway and cell adhesion (Ben-Ze'ev et al., 1998). This interaction between Glut4 and beta-catenin may shed light on the regulation of insulin-responsive glucose transport since it links the transporter to an important signaling pathway. The alpha-SNAP is an important mediator in the cellular process of intracellular transport (St-Denis, et al., 1998). The finding that alpha-SNAP and Glut4 interact provides a link between glucose transportation and the machinery required to perform movement of the glucose transporter between the outside and the interior of the cell. Glut4 has been demonstrated to interact with tropomyosin 3, a protein involved in muscle contraction (Squire et al., 1998). This interaction may represent a link between glucose uptake and muscle function. Finally, the Glut4 glucose transporter has been shown to interact with a putative protein kinase, MAPKKK6. This enzyme has not been well-characterized, but it was identified by virtue of its ability to bind to another protein kinase (Wang et al., 1998). Once again, this may provide a clue to the mechanism of regulation of glucose transport since Glut4 can interact with another potential signal tranduction mediator.

Using the yeast two-hybrid assay, we have been able to detect three proteins which can bind to Glut4, 14-3-3 zeta, the efp-like protein (KIAA0282) and tankyrase. The 14-3-3 zeta is a signal transduction protein which has been shown to interact specifically with phospherine residues (Thorson et al., 1998). 14-3-3 zeta is part of a pathway that links the insulin receptor molecule at

the cell surface to the Glut4 protein located either in the interior of the cell or also at the cell surface. Interestingly, the same small region of Glut4 that interacts with 14-3-3 zeta has been shown to be phosphorylated on a critical serine residue by the kinase Akt-2 that has also been implicated in glucose uptake (Kupriyanova et al., 1999). The efp-like protein is a putative transcription factor (Orimo et al., 1995). Its function is not well described but the Glut4 protein could influence the transcriptional activation of various genes, some of which might be involved in cellular metabolism. Tankyrase is a known telomere-asscoiated protein (Smith et al., 1998).

Using the yeast two-hybrid assay, we have been able to detect an additional protein, which can bind to Glut4. This protein is called MM-1 and was identified by virtue of its ability to interact with the proto-oncogene c-myc (Mori et al., 1998). Other than this original characterization, there is not much else known about MM-1 but on the basis of the interaction found here, MM-1 may play critical roles in both cancer and diabetes.

Other than this original characterization, there was not much else known about MM-1, however because of its association with Glut4 and its tie to Diabetes, we have used MM-1 in two-hybrid assays. Using the yeast two-hybrid assay, we have identified the large centrosomal protein C-Nap1 as an interactor of MM-1. C-Nap1 was originally identified as a protein that could interact with the Nek2 cell cycle-regulated protein kinase (Fry et al., 1998). The finding that MM-1 can interact with C-Nap1 serves to tie Glut4 and glucose transport in general to the control of the cell cycle. The second protein shown to interact with MM-1 is beta spectrin. Spectrins give flexibility to the cell and also act as a scaffold for other cellular proteins (Grum et al., 1999). Interestingly, we have linked beta spectrin to glucose transport and Diabetes in a previous finding where it was shown that beta spectrin could interact with the vesicle-associated protein IRAP. The finding that MM-1 can bind to beta spectrin further strengthens the argument that beta spectrin plays a role in glucose transport. MM-1 was shown to bind to a third protein, KIAA0477, which has no known function. KIAA0477 was originally isolated from brain but its tissue distribution is not known. The finding that KIAA0477 interacts with MM-1 suggests that KIAA0477 plays a role in glucose transport or in some cellular function associated with vesicular transport.

Another approach to understanding the mechanism of glucose transport has been to use the Glut1 molecule (Hresko et al., 1994), a gene highly related to Glut4 and possessing similar biological function, as a molecular bait in the search for other molecules that can interact with it. Using the yeast two-hybrid assay, we have identified three more proteins which can bind to Glut1: DRAL, HSS and myosin heavy chain. DRAL is a LIM domain-containing protein that is also

known as FHL-2 or SLIM3. DRAL was identified as a protein that was expressed in normal muscle tissue culture cells but was down-regulated in cancerous rhabdomyosarcoma cells (Genini et al., 1997). It is possible that DRAL plays a critical role in the terminal differentiation of muscle cells such as cardiac muscle, and that its misregulation could result in an undifferetiated cancerous phenotype. Glut1 was also shown to interact with HSS or human sperm surface protein. HSS is a testis-specific protein that has no known function (Shankar et al., 1998). It does contain a putative transmembrane domain and a leucine-zipper dimerization domain. Since it interacts with Glut1 and it is presumably membrane-bound, HSS could potentially act with Glut1 or Glut4 to affect glucose transport in the testis. Glut1 has also been demonstrated to interact with a form of the myosin heavy chain. Myosin heavy chain plays a key role in muscle structure and contraction (Eddinger et al., 1998). The interaction between Glut1 and myosin suggests that glucose uptake and muscle function may be interrelated via the association of these two proteins.

Using the yeast two-hybrid assay, we identified dynamin as an interactor of Glut1. Dynamin has been implicated in the movement of glucose transporters via vesicular trafficking and likely plays a critical role in endocytosis (or movement from the cell surface to the interior of the cell) (Kao et al., 1998). Because of dynamin's link to Diabetes and glucose transport, we used it in two-hybrid assays and found two proteins that could interact with it. The first protein is called CALM, and it is a clathrin assembly protein similar to the AP-3 family of adaptor proteins. CALM was originally found in a lymphoid myeloid leukemia cell line containing a chromosome translocation resulting in the fusion of the AF10 gene with CALM (Dreyling et al., 1996). Clathrin and its associated proteins have a long history of involvement in the transport of vesicles from the cell surface to the interior of the cell. The association of dynamin and CALM further supports this role and ties CALM to glucose transport. Dynamin also binds to a proteosome activator subunit termed Psme3. The human Psme3 gene maps to the region of the BRCA1 gene, and its function was deduced by its similarity to the mouse gene that is also referred to as the Ki antigen (Kohda et al., 1998). Since the proteosome is required for the post-translational processing and the specific degradation of certain proteins, the finding that Psme3 can bind to dynamin implies that this type of protease activity may play a key role in glucose transport.

Using the yeast two-hybrid assay, we have identified two more proteins which can bind to Glut1, DRAL/FHL2 and cardiac muscle myosin heavy chain. The first protein, DRAL/FHL2, is a protein shown to be down-regulated in rhabdomyosarcoma (Genini et al., 1997). It is entirely composed of LIM domains, polypeptide motifs that form double zinc fingers and may function by

facilitating binding to nucleic acid or other proteins. The same region of Glut1 has been shown to bind to a cardiac muscle myosin heavy chain (MYSA)(Metzger et al., 1999). The significance of this interaction is unknown with regard to glucose transport, however myosin is known to function in muscle contraction and cell structure.

Using the yeast two-hybrid assay, we have identified three proteins which can bind to Glut1, dynamin, and two proteins of unknown function, KIAA0144 and clone 25204. Dynamin has been implicated in the movement of glucose transporters via vesicular trafficking and likely plays a critical role in endocytosis (or movement from the cell surface to the interior of the cell) (Kao et al., 1998). Although the KIAA0144 gene is uncharacterized to date, the region of it that contacts Glut1 is highly enriched for serine, threonine and proline residues, possibly providing a clue to its function. Other proteins with similar "STP" domains include the extracellular portions of cell surface receptors. Finally, a potential translation product of clone 25204 bears a striking resemblance to a previously identified mouse gene called SEZ-6. This gene was found by virtue of its increased transcript levels in brain tissue following exposure to a seizure producing drug (Shimizu-Nishikawa et al., 1995).

The insulin-regulated membranse-spanning aminopeptidase or IRAP (also known as vp165, gp160 and oxytocinase) co-localizes with the Glut4 transporter in specified endocytic vesicles (Keller et al., 1995; Malide et al., 1997). Since expression of the N-terminal fragment of IRAP has been shown to result in the translocation of Glut4 to the plasma membrane, IRAP is thought to play a key role in glucose transport (Waters et al., 1997). Using the two-hybrid system, we have detected the interaction of IRAP with two proteins, 14-3-3 beta and HSS. The 14-3-3 family of proteins are critical signal transduction proteins that bind to phosphoserine residues (Jin et al., 1996). The interaction of IRAP with 14-3-3 beta strongly suggests that the function of IRAP could be regulated by phosphorylation and by the subsequent binding by 14-3-3 family members. Since IRAP and the Glut4 glucose transporter co-localize in the same intracellular vesicles, it is possible that Glut4 may participate in signal transduction mechanisms mediated by the 14-3-3 proteins such as 14-3-3 beta. IRAP has also been shown to bind to the HSS protein described above. Like Glut1 and Glut4, IRAP is membrane-bound and could potentially bind to HSS in the membrane. This finding points to HSS as playing a role in glucose transport or in other important functions performed in intracellular vesicles.

Using the two-hybrid system, we have detected the interaction of IRAP with two more proteins. The N-terminal portion of IRAP has been shown to interact with SLAP-2. The rabbit

homolog of SLAP-2 has been demonstrated to localize to the sarcolemma or the membrane of muscle cells although its function has not been elucidated (Wigle et al., 1997). SLAP-2 may play a role in vesicular transport or may at least participate in it since it has been shown to be membrane-associated and localizes to both the cell membrane as well as to intracellular stores in the endoplasmic reticulum. The C-terminal extracellular portion of IRAP has been demonstrated to interact with SG2NA. SG2NA is a cell cycle nuclear autoantigen that contains so-called WD-40 repeats that are present in a variety of signal transduction proteins (Muro et al., 1995). Once again, the significance of this interaction is unclear however it is possible that SG2NA binding to IRAP is part of a more complex regulatory mechanism.

Using the two-hybrid system, we have detected the interaction of IRAP with one more protein. The N-terminal portion of IRAP has been shown to interact VAMP-associated protein A (VAP-A or VAP-33). This protein has been implicated in intracellular transport (specifically exocytosis or movement to the cell surface) in *A. californica* and likely plays a similar role in humans (Skehel et al., 1995; Weir et al., 1998).

Using the two-hybrid system, we have detected the interaction of IRAP with three proteins. The N-terminal portion of IRAP has been shown to interact with another signal transduction protein, the zeta polypeptide of protein tyrosine phosphatase. This protein is not well characterized but could play a role in regulating glucose transport by dephosphorylating critical proteins that cause or prevent glucose transport (Nishiwaki et al., 1998). The C-terminal portion of IRAP has been shown to interact with non-erythrocytic beta-spectrin. This protein is thought to be involve in secretion and could play a role in the movement of Glut 4 vesicles through IRAP (Hu et al., 1992). The C-terminus of IRAP has also been shown in the two-hybrid assay to interact with the p85 regulatory subunit of phosphatidylinositol-3 (PI-3) kinase. This protein is a central player in cellular signal transduction and participates in transmitting signals from the outside of the cell into the interior. Interestingly, one of the functions of PI-3 kinase p85 involves the insulin receptor (Martin et al., 1996). Further, it is well known that the movement of Glut 4 between the plasma membrane and the interior of the cell depends on the action of the PI-3 kinase signal transduction pathway since inhibitors of this kinase prevent the cycling of Glut4. We also discovered that PI-3 kinase p85 interacts with tankyrase. Two-hybrid interactions have been detected using the p110 catalytic subunit of PI-3 kinase, and these include interactions with the p85 and p55 subunits of the same enzyme complex as well as non-subunit interactions.

Protein phosphatase 5 (PP5) is a TPR domain containing protein that seems to be part of larger multiprotein complexes that possess several cellular functions (Silverstein et al., 1997). Our two-hybrid studies have confirmed the biochemical interaction between protein phosphatase 5 and Hsp90, and the association between these proteins has been previously demonstrated using biochemical methods. PP5 has also been demonstrated using the two-hybrid assay herein to interact with another related heat shock protein, Hsp89, and also with tankyrase.

Phosphatidyl inositol-3 kinase is a very important signal transduction protein and likely plays a critical role in Glut4-mediated glucose uptake (Shankar et al., 1998). This protein participates in transmitting signals from the outside of the cell into the interior. It is composed of two subunits, the p85 regulatory subunit and the p110 catalytic subunit, and functions by facilitating the transmission of signals from the outside of the cell into the interior. PI-3 kinase has been implicated in insulin-regulation and glucose uptake since one of its functions involves the insulin receptor (Martin et al., 1996). Further, the movement of Glut4 between the plasma membrane and the interior of the cell depends on the action of the PI-3 kinase signal transduction pathway since inhibitors of this kinase prevent the cycling of Glut4.

The p85 regulatory subunit of PI-3 kinase has been shown to interact with several proteins in the two-hybrid assay. Here we report the identification of five more proteins that can interact with p85. SLP-76 is a tyrosine phosphoprotein that participates in T cell signaling (Clements et al., 1998; Jackman et al., 1995). It is thought that SLP-76 acts as a so-called adaptor protein since it plays a role in intermediate steps of signal transduction. This is achieved by bridging factors that act at the plasma membrane with other molecules that perform functions within the interior of the cell. Our results indicate that SLP-76 may play a critical role in the PI-3 kinase signal transduction pathway by virtue of its ability to bind the p85 regulatory subunit. The p85 subunit of PI-3 kinase has also been demonstrated to bind to two more important signal transduction proteins: 14-3-3 zeta and 14-3-3 eta. These proteins bind specifically to phosphoserine residues in a number of proteins (Oghira et al., 1997; Thorson et al., 1998; Yaffe et al., 1997). Interestingly, our studies have shown that the Glut4 glucose transporter can also interact with 14-3-3 zeta. Thus, PI-3 kinase can also be connected to glucose uptake mechanisms by its interaction with the 14-3-3 signal transduction proteins. PI-3 kinase p85 was shown to interact with chromogranin C, a neuroendocrine secretory granule protein in the granin family (Ozawa et al., 1995). Members of the granin family localize to specialized secretory vesicles and are thought to serve an important function in protein sorting and secretion (Leitner et al., 1999). Finally, TACC2 has been shown to interact with PI-3 kinase

p85 in the yeast two-hybrid assay. TACC2 is a member of a family of "transforming coiled coil" proteins that have been implicated in cellular growth control and cancer (Still et al., 1999). Although the function of TACC2 remains unknown, its interaction with p85 demonstrates that it may also be a part of an important signal transduction pathway.

The p110 subunit of this protein has been shown to interact with complement protein C4, tenascin XB and alpha acid glucosidase (GAA). The complement C4 protein plays a key role in acitvating the classical complement pathway, and it is involved in evoking histamine release from basophils and mast cells. Naturally occurring deficiencies of C4 have been correlated with a number of immune-related human diseases such as Systemic Lupus Erythmatosus, kidney disease, hepatitis, dementia and the propensity for recurrent infectiions (Mascart-Lemone et al., 1983; Vergani et al., 1985; Waters et al., 1997; Nerl et al., 1984; Lhotta et al., 1990). The finding that C4 interacts with the p110 subunit of PI-3 kinase suggests that the function of C4 is somehow regulated by this signal transduction, perhaps in response to some immunologic stimulus. The p110 catalytic subunit of PI-3 kinase has been demonstrated to interact with tenascin XB. Tenascin XB is an extracellular structural protein. Deficiency of tenascin XB is linked to the connective tissue disorder Ehler-Danlos syndrome (Burch et al., 1997). This finding that p110 can bind to tenascin XB suggest that the function of tenascin XB could be modified or regulated by the PI-3 kinase signal trasduction pathway. PI-3 kinase p110 has also been shown to bind to GAA or alph acid glucosidase. GAA is a lysosomal enzyme that catalyzes the release of glucose from glycosylated substrates, and defects in GAA result in a glycogen storage disease (Raben et al., 1995). The finding that PI-3 kinase can bind to GAA suggests that GAA activity may be influenced by the PI-3 kinase signaling mechanism. Our previous two-hybrid results have linked the PI-3 kinase to human diseases such as Diabetes and Alzheimer's disease. These new findings therefore link complement protein C4, tenascin XB and alpha acid glucosidase to these diseases as well as to the other diseases already described.

The p110 subunit of this protein has been shown to interact with the thyroid hormone interacting protein TRIP15 in the two-hybrid assay. TRIP15 is part of a larger multiprotein complex termed the signalsome that is likely to be involved in cell signaling (Seeger et al., 1998). Our two-hybrid results have linked the PI-3 kinase to human diseases such as Diabetes and Alzheimer's disease.

We have detected the interaction of PI-3 kinase p110 subunit with the β-amyloid precursor protein (APP). In brief, there is now growing evidence that APP metabolism and Aβ generation are

central events to AD pathogenesis. For a more extensive review of APP and AD pathogenesis, see U.S. patent application Serial No. 09/466,139, filed 21 December 1999 and international patent application No. PCT/US99/30396, filed 21 December 1999, each incorporated herein by reference. Although several candidates (cathepsin G, cathepsin D, chymotrypsin, and others) have been suggested, the β-secretase enzyme has not yet been identified. Even less is known about α- and γ-secretases. The biochemical link between the presenilins and APP processing has not been established. The proteins that mediate the neurotrophic and neuroprotective effects of sAPP are unknown. This last point is of utmost importance because an alteration of APP metabolism could result in both the generation of a toxic product (Aβ) and the impairment of sAPP trophic activity (Saitoh et al.1994; Roch et al.1993; Saitoh and Roch, 1995). In this respect, it is interesting that one APP mutation associated with Alzheimer's results in a defective neurite extension activity of sAPP (Li et al., 1997). Moreover, the balance of phosphorylation cascades is deeply altered in Alzheimer brains (Saitoh and Roch, 1995; Jin and Saitoh, 1995; Mook-Jung and Saitoh, 1997; Saitoh et al., 1991; Shapiro et al., 1991). Since APP is thought to play a major role in the pathology of Alzheimer's disease, the elucidation of a tie between PI-3 kinase and APP provides a new target for discovering the treatment for this neurological disorder (Russo et al., 1998).

O-linked N-acetylglucosaminyltransferase or OGTase is an enzyme implicated in intracellular signal transduction (Kreppel et al., 1997). It has been speculated that OGTase may play a key role in glucose uptake and may therefore participate in the Diabetes related pathways (Cooksey et al., 1999). OGTase has been shown to also interact with myosin heavy chain. The binding of myosin heavy chain to OGTase suggests that the function of myosin in muscle structure or function could be influenced by OGTase in its signal transductive capacity. This could potentially affect many cellular processes in the muscle cell, including glucose transport. OGTase has been shown to bind to a novel protein termed PN6931. PN6931 is very similar to the mouse kinesin light chain gene (GenBank accession AF055666). Kinesin is a molecular motor involved in cellular transport and chromosome movement (Kirchner et al., 1999). Perhaps by post-translationally modifying this novel kinesin-like protein, OGTase can influence its function.

Amino acids 250 to 450 of OGTase has been shown to interact with a member of the 14-3-3 protein family, 14-3-3 epsilon. The 14-3-3 proteins function in intracellular signal transduction pathways by specifically binding to phosphoserine residues on other critical signaling molecules (Ogihara et al., 1997; Yaffe et al., 1997). The interaction between OGTase and 14-3-3 epsilon may

serve to link two different signal transduction pathways, one which involves phosphorylation and a second which involves another type of protein modification.

Amino acids 250 to 450 of OGTase has been shown to interact with two proteins, alpha-2 catenin and Nafl a. Alpha-catenin is a protein related to vinculins that functions in cell-cell contact by binding to cadherins (Rudiger et al., 1998). The same region of OGTase can interact with Nafl b, a protein identified by virtue of its ability to bind the Nef gene product of HIV in the two-hybrid assay (Fukushi et al., 1999). Over-expression of Nafl a was observed to cause an increase in cell surface expression of the CD4 antigen, therefore this protein may also function in intracellular trafficking and could potentially participate in a number of diseases related to this general process such as Diabetes and Alzheimer's.

Using the yeast two-hybrid assay, we identified Nafl b as an interactor of OGTase. Nafl b is a protein which was identified by virtue of its ability to bind the Nef gene product of HIV in the two-hybrid assay (Fukushi et al., 1999). Over-expression of Nafl b was observed to cause an increase in cell surface expression of the CD4 antigen, therefore this protein may also function in intracellular trafficking and could potentially participate in a number of diseases related to this general process such as Diabetes and Alzheimer's. In a two-hybrid search using Nafl b as a bait, the TRAF-interacting protein I-TRAF was found to be an interactor. I-TRAF appears to act as a regulator of the TRAF signal transduction pathway that transmits signals from TNF (tumor necrosis factor) receptor family members (Rothe et al., 1996). The observation that Nafl b interacts with I-TRAF serves to link an extracellular stimulated signal transduction mechanism with the OGTase pathway involved in glucose transport. A two-hybrid search using Nafl b as a bait has also identified a novel protein fragment called PN7582. This small protein fragment does not appear to have any strong similarity to known proteins therefore its function is not readily apparent. It is very possible that it may participate in protein trafficking or signal transduction by its association with Nafl b.

In a two-hybrid search using OGTase as a bait, four new interactors were found. The first interactor, desmin, is a cytoplasmic intermediate filament protein found in muscle (Capetanaki et al., 1998). It functions in striated muscle by connecting myofibrils with themselves and with the plasma membrane. Desmin is broken down into three functional domains, the head, rod and tail, and OGTase can bind to the rod structure. The second protein shown to interact with OGTase is called alpha-karyopherin. Alpha-karyopherin (also known as importin and SRP1) is a ubiquitously expressed protein that plays a role in trafficking nuclear localization signal-containing proteins into

the nucleus through the nuclear pore (Moroianu, 1997). The third protein that OGTase has been shown to bind is glutanimyl-tRNA synthetase. The aminoacyl-tRNA synthetases not only play a key role in protein synthesis, but recent studies have shown that they also impact a number of other cellular processes such as tRNA processing, RNA splicing, RNA trafficking, apoptosis, and transcriptional and translational regulation (Martinis et al., 1999). The fourth protein shown to bind to OGTase in the yeast two-hybrid assay is called clone 25100 and it has no known function. The gene for clone 25100 was isolated from human infant brain and appears to encode a small protein with no structural characteristics that shed light on its function. All four of these OGTase-interacting proteins may act as substrates for OGTase or may affect its function in some way. The finding that they interact with OGTase suggests that they may play a role in glucose transport or in the pathogenesis of Diabetes.

Our yeast two-hybrid studies have demonstrated that OGTase can interact with a variety of proteins that can fall into a number of functional categories. Two proteins that have been implicated in vesicular transport bind to OGTase. The first is called BAP31, and it likely plays a critical role in sorting and transporting membrane proteins between intracellular compartments (Annaert et al., 1997). The second protein is called dynamin II, and it is implicated in the movement of transport vesicles from the plasma membrane to sites within the interior of the cell (Sontag et al., 1994). OGTase has been demonstrated to interact with two proteins that function in transcription, as well. The first is called EGR1 for early growth response protein 1, and it has been shown to be highly induced in cells following mitogenic stimulation (Sukhatme et al., 1988). It is a zinc-finger containing protein, and following its own stimulation, goes on to activate the transcription of genes involved in mitogenesis and differentiation. Additionally, OGTase can interact with MOP2, a basic helix-loop-helix containing transcription factor that is involved in the induction of oxygen regulated genes (Hogenesch et al., 1997). OGTase binds to a structural protein called talin. Talin is a cytoplasmic protein that serves to link integrins with the actin cytoskeleton (Calderwood etal., 1999). Finally, OGTase has been shown in the yeast two-hybrid assay to bind to five proteins of unknown or poorly characterized function. OGTase binds to Int-6, a protein that has also been demonstrated to bind to the HTLV-I Tax transactivator and it is a component of promyelocytic leukemia nuclear bodies (Desbois et al., 1996). OGTase binds to interferon-induced protein 54, an uncharacterized protein that shows a large increase in its transcript following treatment with alpha- and beta-interferons (Levy et al., 1986). Lastly, HSPC028, KIAA0443, and clone 25100 interact with OGTase.

Akt kinase is a serine/threonine protein kinase that has been implicated in insulin-regulated glucose transport and the development of non-insulin dependent diabetes mellitus (Krook et al., 1998). Because of this link, Akt kinase has been used in two-hybrid assays to determine what proteins interact with it either because they are substrates of Akt kinase or because they are regulators of the kinase. Two closely related Akt proteins were used: Akt1 and its closely related family-member Akt2. Akt1 and Akt2 were both shown to interact with the nuclear mitotic apparatus protein NuMA1. NuMA1 displays a distinct pattern of immunofluorescent staining that varies throughout the cell cycle. It is nuclear throughout interphase but re-localizes to the spindle apparatus during mitosis (Lydersen et al., 1980). Phosphorylation is thought to play a critical role in NuMA function. It appears to become phosphorylated just prior to mitosis and becomes dephosphorylated after mitosis has occurred in the G1 phase of the cell cycle mitosis (Sparks et al., 1995). Akt1 and Akt2 may be capable of phosphorylating NuMA, especially since the region of NuMA that interacts with Akt2 (amino acids 98 to 365) contains 23 serines and 9 threonines. Akt2 was also shown to interact with two proteins involved in vesicular transport. The first protein, BAP31, likely plays a role in the movement of membrane-bound proteins from the Golgi appartus to the plasma membrane (Annaert et al., 1997). Since BAP31 was also shown to interact with OGTase in our previous experiments, two signal transduction proteins implicated in glucose transport have been tied to BAP31. Akt2 was also shown to bind to another vesicular transport protein termed beta-adaptin or AP2-beta. Beta-adaptin is a part of the AP2 coat assembly complex that links clathrin and to transmembrane receptors resident in coated vesicles (Pearse, 1989). This finding suggests a role for Akt2 in the regulation of endocytosis, while the finding that Akt2 binds to BAP31 provides for a tie between Akt2 and exocytosis. All of the proteins that can interact with the Akt kinase may play a role in glucose transport by virtue of their association with Akt.

PTP1b is a protein tyrosine phosphatase that plays a critical role in signal transduction. It has been implicated as a negative regulator of insulin-responsive glucose transport (Chen et al., 1997), and therefore it has been used in yeast two-hybrid assays in an attempt to find more proteins involved in this function, perhaps by acting as substrates. PTP1b was shown to interact with VAMP-associated protein A (VAP-A or VAP-33). This protein has been implicated in intracellular transport (specifically exocytosis or movement to the cell surface) in *A. californica* and likely plays a similar role in humans (Skehel et al., 1995; weir et al., 1998). The interaction between PTP1b and VAP-A serves as a potential tie between PTP1b and IRAP since IRAP was also shown to bind to VAP-A in two-hybrid experiments. IRAP, insulin-regulated membrane-spanning aminopeptidase

(also known as vp165, gp160 and oxytocinase) co-localizes with the Glut4 transporter in specified endocytic vesicles (Keller et al., 1995; Malide et al., 1997). Since expression of the N-terminal fragment of IRAP has been shown to result in the translocation of Glut4 to the plasma membrane, IRAP is thought to play a key role in glucose transport (Waters et al., 1997). Thus, the result that PTP1b interacts with VAP-A serves to strengthen the tie between PTP1b and VAP-A to glucose transport and Diabetes.

The small GTP-binding protein Rab4 is another signal transduction factor that has been implicated in the regulation of insulin-stimulated glucose uptake (Vollenweider et al., 1997; Cormont et al., 1996). Rab4, therefore, has also been used in the yeast two-hybrid assay to find additional proteins involved in glucose transport and in Diabetes. Two proteins have been shown to bind to Rab4, an alpha-catenin-like protein (sometimes called alpha-catulin) and another small GTP-binding protein called Rab2. The alpha-catenin-like protein resembles alpha-catenin and vinculin however its function has not yet been well-characterized (Janssens et al., 1999). Alpha-catenin itself is a protein related to vinculins that functions in cell-cell contact by binding to cadherins (Rudinger et al., 1998). Interestingly, alpha-catenin was found in our previous studies to bind to OGTase and, as a consequence, has been implicated in glucose transport. The second protein shown to bind to Rab4 is Rab2. These two small GTP-binding proteins are highly related with a 50% amino acid identity. Unlike Rab4, Rab2 has not been thought to play a role in insulin-stimulated glucose transport (Uphues et al., 1994) although it does play an important role in vesicular transport in the cell (Tisdale et al., 1998). The finding that Rab4 and Rab2 interact suggests that they may be capable of influencing each others cellular functions, thus Rab2 could potentially affect Rab4's role in glucose transport.

The proteins disclosed in the present invention were found to interact with their corresponding proteins in the yeast two-hybrid system. Because of the involvement of the corresponding proteins in the physiological pathways disclosed herein, the proteins disclosed herein also participate in the same physiological pathways. Therefore, the present invention provides a list of uses of these proteins and DNA encoding these proteins for the development of diagnostic and therapeutic tools useful in the physiological pathways. This list includes, but is not limited to, the following examples.

Two-Hybrid System

The principles and methods of the yeast two-hybrid system have been described in detail elsewhere (e.g., Bartel and Fields, 1997; Bartel et al., 1993; Fields and Song, 1989; Chevray and Nathans, 1992). The following is a description of the use of this system to identify proteins that interact with a protein of interest.

The target protein is expressed in yeast as a fusion to the DNA-binding domain of the yeast Gal4p. DNA encoding the target protein or a fragment of this protein is amplified from cDNA by PCR or prepared from an available clone. The resulting DNA fragment is cloned by ligation or recombination into a DNA-binding domain vector (e.g., pGBT9, pGBT.C, pAS2-1) such that an in-frame fusion between the Gal4p and target protein sequences is created.

The target gene construct is introduced, by transformation, into a haploid yeast strain. A library of activation domain fusions (i.e., adult brain cDNA cloned into an activation domain vector) is introduced by transformation into a haploid yeast strain of the opposite mating type. The yeast strain that carries the activation domain constructs contains one or more Gal4p-responsive reporter gene(s), whose expression can be monitored. Examples of some yeast reporter strains include Y190, PJ69, and CBY14a. An aliquot of yeast carrying the target gene construct is combined with an aliquot of yeast carrying the activation domain library. The two yeast strains mate to form diploid yeast and are plated on media that selects for expression of one or more Gal4p-responsive reporter genes. Colonies that arise after incubation are selected for further characterization.

The activation domain plasmid is isolated from each colony obtained in the two-hybrid search. The sequence of the insert in this construct is obtained by the dideoxy nucleotide chain termination method. Sequence information is used to identify the gene/protein encoded by the activation domain insert via analysis of the public nucleotide and protein databases. Interaction of the activation domain fusion with the target protein is confirmed by testing for the specificity of the interaction. The activation domain construct is co-transformed into a yeast reporter strain with either the original target protein construct or a variety of other DNA-binding domain constructs. Expression of the reporter genes in the presence of the target protein but not with other test proteins indicates that the interaction is genuine.

In addition to the yeast two-hybrid system, other genetic methodologies are available for the discovery or detection of protein-protein interactions. For example, a mammalian two-hybrid system is available commercially (Clontech, Inc.) that operates on the same principle as the yeast two-hybrid system. Instead of transforming a yeast reporter strain, plasmids encoding DNA-binding and

activation domain fusions are transfected along with an appropriate reporter gene (e.g., lacZ) into a mammalian tissue culture cell line. Because transcription factors such as the *Saccharomyces cerevisiae* Gal4p are functional in a variety of different eukaryotic cell types, it would be expected that a two-hybrid assay could be performed in virtually any cell line of eukaryotic origin (e.g., insect cells (SF9), fungal cells, worm cells, etc.). Other genetic systems for the detection of protein-protein interactions include the so-called SOS recruitment system (Aronheim et al., 1997).

Protein-protein interactions

Protein interactions are detected in various systems including the yeast two-hybrid system, affinity chromatography, co-immunoprecipitation, subcellular fractionation and isolation of large molecular complexes. Each of these method is well characterized and can be readily performed by one skilled in the art. See, e.g., U.S. Patents No. 5,622,852 and 5,773,218, PCT published application No. WO 97/27296 and PCT published application No. WO 99/65939, each of which are incorporated herein by reference.

The protein of interest (or an interacting domain thereof) can be produced in eukaryotic or prokaryotic systems. A cDNA encoding the desired protein is introduced in an appropriate expression vector and transfected in a host cell (which could be bacteria, yeast cells, insect cells, or mammalian cells). Purification of the expressed protein is achieved by conventional biochemical and immunochemical methods well known to those skilled in the art. The purified protein is then used for affinity chromatography studies: it is immobilized on a matrix and loaded on a column. Extracts from cultured cells or homogenized tissue samples are then loaded on the column in appropriate buffer, and non-binding proteins are eluted. After extensive washing, binding proteins or protein complexes are eluted using various methods such as a gradient of pH or a gradient of salt concentration. Eluted proteins can then be separated by two-dimensional gel electrophoresis, eluted from the gel, and identified by micro-sequencing. The purified proteins can also be used for affinity chromatography to purify interacting proteins disclosed herein. All of these methods are well known to those skilled in the art.

Similarly, both proteins of the complex of interest (or interacting domains thereof) can be produced in eukaryotic or prokaryotic systems. The proteins (or interacting domains) can be under control of separate promoters or can be produced as a fusion protein. The fusion protein may include a peptide linker between the proteins (or interacting domains) which, in one embodiment,

serves to promote the interaction of the proteins (or interacting domains). All of these methods are also well known to those skilled in the art.

Purified proteins of interest, individually or a complex, can also be used to generate antibodies in rabbit, mouse, rat, chicken, goat, sheep, pig, guinea pig, bovine, and horse. The methods used for antibody generation and characterization are well known to those skilled in the art. Monoclonal antibodies are also generated by conventional techniques. Single chain antibodies are further produced by conventional techniques.

DNA molecules encoding proteins of interest can be inserted in the appropriate expression vector and used for transfection of eukaryotic cells such as bacteria, yeast, insect cells, or mammalian cells, following methods well known to those skilled in the art. Transfected cells expressing both proteins of interest are then lysed in appropriate conditions, one of the two proteins is immunoprecipitated using a specific antibody, and analyzed by polyacrylamide gel electrophoresis. The presence of the binding protein (co-immunoprecipitated) is detected by immunoblotting using an antibody directed against the other protein. Co-immunoprecipitation is a method well known to those skilled in the art.

Transfected eukaryotic cells or biological tissue samples can be homogenized and fractionated in appropriate conditions that will separate the different cellular components. Typically, cell lysates are run on sucrose gradients, or other materials that will separate cellular components based on size and density. Subcellular fractions are analyzed for the presence of proteins of interest with appropriate antibodies, using immunoblotting or immunoprecipitation methods. These methods are all well known to those skilled in the art.

Disruption of protein-protein interactions

It is conceivable that agents that disrupt protein-protein interactions can be beneficial in many physiological disorders, including, but not-limited to NIDDM, AD and others disclosed herein. Each of the methods described above for the detection of a positive protein-protein interaction can also be used to identify drugs that will disrupt said interaction. As an example, cells transfected with DNAs coding for proteins of interest can be treated with various drugs, and co-immunoprecipitations can be performed. Alternatively, a derivative of the yeast two-hybrid system, called the reverse yeast two-hybrid system (Leanna and Hannink, 1996), can be used, provided that the two proteins interact in the straight yeast two-hybrid system.

Modulation of protein-protein interactions

Since the interaction described herein is involved in a physiological pathway, the identification of agents which are capable of modulating the interaction will provide agents which can be used to track the physiological disorder or to use as lead compounds for development of therapeutic agents. An agent may modulate expression of the genes of interacting proteins, thus affecting interaction of the proteins. Alternatively, the agent may modulate the interaction of the proteins. The agent may modulate the interaction of wild-type with wild-type proteins, wild-type with mutant proteins, or mutant with mutant proteins. Agents can be tested using transfected host cells, cell lines, cell models or animals, such as described herein, by techniques well known to those of ordinary skill in the art, such as disclosed in U.S. Patents No. 5,622,852 and 5,773,218, PCT published application No. WO 97/27296 and PCT published application No. WO 99/65939, each of which are incorporated herein by reference. The modulating effect of the agent can be screened *in vivo* or *in vitro*. Exemplary of a method to screen agents is to measure the effect that the agent has on the formation of the protein complex.

Mutation screening

The proteins disclosed in the present invention interact with one or more proteins known to be involved in a physiological pathway, such as in NIDDM or AD. Mutations in interacting proteins could also be involved in the development of the physiological disorder, such as NIDDM or AD, for example, through a modification of protein-protein interaction, or a modification of enzymatic activity, modification of receptor activity, or through an unknown mechanism. Therefore, mutations can be found by sequencing the genes for the proteins of interest in patients having the physiological disorder, such as insulin, and non-affected controls. A mutation in these genes, especially in that portion of the gene involved in protein interactions in the physiological pathway, can be used as a diagnostic tool, and the mechanistic understanding the mutation provides can help develop a therapeutic tool.

Screening for at-risk individuals

Individuals can be screened to identify those at risk by screening for mutations in the protein disclosed herein and identified as described above. Alternatively, individuals can be screened by analyzing the ability of the proteins of said individual disclosed herein to form natural complexes. Further, individuals can be screened by analyzing the levels of the complexes or individual proteins

of the complexes or the mRNA encoding the protein members of the complexes. Techniques to detect the formation of complexes, including those described above, are known to those skilled in the art. Techniques and methods to detect mutations are well known to those skilled in the art. Techniques to detect the level of the complexes, proteins or mRNA are well known to those skilled in the art.

Cellular models of Physiological Disorders

A number of cellular models of many physiological disorders or diseases have been generated. The presence and the use of these models are familiar to those skilled in the art. As an example, primary cell cultures or established cell lines can be transfected with expression vectors encoding the proteins of interest, either wild-type proteins or mutant proteins. The effect of the proteins disclosed herein on parameters relevant to their particular physiological disorder or disease can be readily measured. Furthermore, these cellular systems can be used to screen drugs that will influence those parameters, and thus be potential therapeutic tools for the particular physiological disorder or disease. Alternatively, instead of transfecting the DNA encoding the protein of interest, the purified protein of interest can be added to the culture medium of the cells under examination, and the relevant parameters measured.

Animal models

The DNA encoding the protein of interest can be used to create animals that overexpress said protein, with wild-type or mutant sequences (such animals are referred to as "transgenic"), or animals which do not express the native gene but express the gene of a second animal (referred to as "transplacement"), or animals that do not express said protein (referred to as "knock-out"). The knock-out animal may be an animal in which the gene is knocked out at a determined time. The generation of transgenic, transplacement and knock-out animals (normal and conditioned) uses methods well known to those skilled in the art.

In these animals, parameters relevant to the particular physiological disorder can be measured. These parametes may include receptor function, protein secretion *in vivo* or *in vitro*, survival rate of cultured cells, concentration of particular protein in tissue homogenates, signal transduction, behavioral analysis, protein synthesis, cell cycle regulation, transport of compounds across cell or nuclear membranes, enzyme activity, oxidative stress, production of pathological products, and the like. The measurements of biochemical and pathological parameters, and of

behavioral parameters, where appropriate, are performed using methods well known to those skilled in the art. These transgenic, transplacement and knock-out animals can also be used to screen drugs that may influence the biochemical, pathological, and behavioral parameters relevant to the particular physiological disorder being studied. Cell lines can also be derived from these animals for use as cellular models of the physiological disorder, or in drug screening.

Rational drug design

The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact (e.g., agonists, antagonists, inhibitors) in order to fashion drugs which are, for example, more active or stable forms of the polypeptide, or which, e.g., enhance or interfere with the function of a polypeptide *in vivo*. Several approaches for use in rational drug design include analysis of three-dimensional structure, alanine scans, molecular modeling and use of anti-id antibodies. These techniques are well known to those skilled in the art.

Following identification of a substance which modulates or affects polypeptide activity, the substance may be further investigated. Furthermore, it may be manufactured and/or used in preparation, i.e., manufacture or formulation, or a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals.

A substance identified as a modulator of polypeptide function may be peptide or non-peptide in nature. Non-peptide "small molecules" are often preferred for many *in vivo* pharmaceutical uses. Accordingly, a mimetic or mimic of the substance (particularly if a peptide) may be designed for pharmaceutical use.

The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This approach might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g., pure peptides are unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing is generally used to avoid randomly screening large numbers of molecules for a target property.

Once the pharmacophore has been found, its structure is modeled according to its physical properties, e.g., stereochemistry, bonding, size and/or charge, using data from a range of sources, e.g., spectroscopic techniques, x-ray diffraction data and NMR. Computational analysis, similarity

mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modeling process.

A template molecule is then selected, onto which chemical groups that mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted thereon can be conveniently selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, and does not degrade *in vivo*, while retaining the biological activity of the lead compound. Alternatively, where the mimetic is peptide-based, further stability can be achieved by cyclizing the peptide, increasing its rigidity. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent it is exhibited. Further optimization or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

Diagnostic Assays

The identification of the interactions disclosed herein enables the development of diagnostic assays and kits, which can be used to determine a predisposition to or the existence of a physiological disorder. In one aspect, one of the proteins of the interaction is used to detect the presence of a "normal" second protein (i.e., normal with respect to its ability to interact with the first protein) in a cell extract or a biological fluid, and further, if desired, to detect the quantitative level of the second protein in the extract or biological fluid. The absence of the "normal" second protein would be indicative of a predisposition or existence of the physiological disorder. In a second aspect, an antibody against the protein complex is used to detect the presence and/or quantitative level of the protein complex. The absence of the protein complex would be indicative of a predisposition or existence of the physiological disorder.

EXAMPLES

The present invention is further detailed in the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized.

EXAMPLE 1

Yeast Two-Hybrid System

The principles and methods of the yeast two-hybrid systems have been described in detail (Bartel and Fields, 1997). The following is thus a description of the particular procedure that we used, which was applied to all proteins.

The cDNA encoding the bait protein was generated by PCR from brain cDNA. Gene-specific primers were synthesized with appropriate tails added at their 5' ends to allow recombination into the vector pGBTQ. The tail for the forward primer was 5'-GCAGGAAACAGCTATGACCATACAGTCAGCGGCCGCCACC-3' (SEQ ID NO:1) and the tail for the reverse primer was 5'-ACGGCCAGTCGCGTGGAGTGTTATGTCATGCGGCCGCTA-3' (SEQ ID NO:2). The tailed PCR product was then introduced by recombination into the yeast expression vector pGBTQ, which is a close derivative of pGBTC (Bartel et al., 1996) in which the polylinker site has been modified to include M13 sequencing sites. The new construct was selected directly in the yeast J693 for its ability to drive tryptophane synthesis (genotype of this strain: Mat α, ade2, his3, leu2, trp1, URA3::GAL1-lacZ LYS2::GAL1-HIS3 gal4del gal80del cyhR2). In these yeast cells, the bait is produced as a C-terminal fusion protein with the DNA binding domain of the transcription factor Gal4 (amino acids 1 to 147). A total human brain (37 year-old male Caucasian) cDNA library cloned into the yeast expression vector pACT2 was purchased from Clontech (human brain MATCHMAKER cDNA, cat. # HL4004AH), transformed into the yeast strain J692 (genotype of this strain: Mat a, ade2, his3, leu2, trp1, URA3::GAL1-lacZ LYS2::GAL1-HIS3 gal4del gal80del cyhR2), and selected for the ability to drive leucine synthesis. In these yeast cells, each cDNA is expressed as a fusion protein with the transcription activation domain of the transcription factor Gal4 (amino acids 768 to 881) and a 9 amino acid hemagglutinin epitope tag. J693 cells (Mat α type) expressing the bait were then mated with J692 cells (Mat a type) expressing proteins from the brain library. The resulting diploid yeast cells expressing proteins interacting with the bait protein were selected for the ability to synthesize tryptophane, leucine, histidine, and β-galactosidase. DNA was prepared from each clone, transformed by electroporation into *E. coli* strain KC8 (Clontech KC8 electrocompetent cells, cat # C2023-1), and the cells were selected on ampicillin-containing plates in the absence of either tryptophane (selection for the bait plasmid) or leucine (selection for the brain library plasmid). DNA for both plasmids was prepared and sequenced by di-deoxynucleotide chain termination method. The identity of the bait cDNA insert was confirmed and the cDNA insert from the brain library plasmid was identified using BLAST program against public

nucleotides and protein databases. Plasmids from the brain library (preys) were then individually transformed into yeast cells together with a plasmid driving the synthesis of lamin fused to the Gal4 DNA binding domain. Clones that gave a positive signal after β-galactosidase assay were considered false-positives and discarded. Plasmids for the remaining clones were transformed into yeast cells together with plasmid for the original bait. Clones that gave a positive signal after β-galactosidase assay were considered true positives.

EXAMPLE 2

Identification of Glut4/CARP Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 463-509 of Glut4 (Swiss Protein (SP) accession no. P14672) as bait was performed. One clone that was identified by this procedure included the amino acids encoded by nucleotides 312-1155 of CARP (GenBank (GB) accession no. X83703).

EXAMPLE 3

Identification of GLUT1/DRAL(FHL2) Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 448-492 of Glut1 (SP accession no. P11166) as bait was performed. One clone that was identified by this procedure included amino acids 1-279 of DRAL(FHL2) (SP accession no. Q13229).

EXAMPLE 4

Identification of GLUT1/Myosin Heavy Chain Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 448-492 of Glut1 (SP accession no. P11166) as bait was performed. One clone that was identified by this procedure included amino acids 1589-1909 of myosin heavy chain (SP accession no. P13533).

EXAMPLE 5

Identification of GLUT1/HSS Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 448-492 of Glut1 (SP accession no. P11166) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 1-? of HSS (GB accession no. X91879).

EXAMPLE 6

Identification of OGTase/Myosin Heavy Chain Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (GB accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 1773-2283 of myosin heavy chain (GB accession no. AF111785).

EXAMPLE 7

Identification of IRAP/14-3-3 beta Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 449-880 of IRAP (GB accession no. U62768) as bait was performed. One clone that was identified by this procedure included amino acids 97-236 of 14-3-3 beta (SP accession no. P31946).

EXAMPLE 8

Identification of IRAP/HSS Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 62-880 of IRAP (GB accession no. U62768) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 726-1446 of HSS (GB accession no. X91879).

EXAMPLE 9

Identification of PI-3K110/Complement Protein C4 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 1-300 of PI-3 kinase p110 subunit (SP accession no. P42338) as bait was performed. One clone that was identified by this procedure included amino acids 1056-1277 of complementj protein C4 (SP accession no. P01028).

EXAMPLE 10

Identification of PI-3K110/Tenascin XB Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 1-300 of PI-3 kinase p110 subunit (SP accession no. P42338) as bait was performed. One clone that was

identified by this procedure included amino acids 3573-3787 of tenascin XB (SP accession no. P78530).

EXAMPLE 11

Identification of PI-3K110/GAA Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 1-300 of PI-3 kinase p110 subunit (SP accession no. P42338) as bait was performed. One clone that was identified by this procedure included amino acids 513-? of GAA (SP accession no. P10253).

EXAMPLE 12

Identification of MM-1/C-Nap1 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 27-175 of MM-1 (SP accession no. Q99471) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 1508-1949 of C-Nap1 (GB accession no. AF049105).

EXAMPLE 13

Identification of MM-1/Beta Spectrin Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 1-175 of MM-1 (SP accession no. Q99471) as bait was performed. One clone that was identified by this procedure included amino acids 1545-1789 of beta spectrin (SP accession no. Q01082).

EXAMPLE 14

Identification of MM-1/KIAA0477 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 27-175 of MM-1 (SP accession no. Q99471) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 2448-3207 of KIAA0477 (GB accession no. AB007946).

EXAMPLE 15

Identification of Dynamin/CALM Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 250-700 of dynamin (SP accession no. Q05193) as bait was performed. One clone that was identified by this

procedure included amino acids encoded by nucleotides 948-1599 of CALM (GB accession no. U45976).

EXAMPLE 16

Identification of Dynamin/Psme3 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 250-700 of dynamin (SP accession no. Q05193) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 378-966 of Psme3 (GB accession no. U11292).

EXAMPLE 17

Identification of Naf1b/I-TRAF Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 590-1610 of Naf1b (GB accession no. Q05193) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 209-1420 of I-TRAF (GB accession no. U59863).

EXAMPLE 18

Identification of Akt1/NuMA1 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 1-118 of Akt1 (SP accession no. P31749) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 813-1341 of NuMA1 (GB accession no. Z11584).

EXAMPLE 19

Identification of Akt2/NuMA1 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 1-152 of Akt2 (SP accession no. P31751) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 552-1353 of NuMA1 (GB accession no. Z11584).

EXAMPLE 20

Identification of Akt2/BAP31 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 915-1311 of Akt2 (GB accession no. M95936) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 469-877 of BAP31 (GB accession no. NM00574).

EXAMPLE 21

Identification of Akt2/Beta Adaptin Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 915-1311 of Akt2 (GB accession no. M95936) as bait was performed. One clone that was identified by this procedure included amino acids 214-594 of beta adaptin (SP accession no. P21851).

EXAMPLE 22

Identification of OGTase/Desmin Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (GB accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids 219-449 of desmin (SP accession no. P17661).

EXAMPLE 23

Identification of OGTase/Alpha-karyopherin Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (GB accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids 152-444 of alpha-karyopherin (SP accession no. P52294).

EXAMPLE 24

Identification of OGTase/Glutaminyl tRNA Synthetase Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (GB accession no. U77413) as bait was performed. One clone

that was identified by this procedure included amino acids 36-161 of glutaminyl tRNA synthetase (SP accession no. P47897).

EXAMPLE 25

Identification of OGTase/Clone 25100 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (GB accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids 11-338 of clone 25100 (GB accession no. AF131780).

EXAMPLE 26

Identification of PTP1b/VAP-A Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 280-436of PTP1b (SP accession no. P18031) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 9-? of VAP-A (GB accession no. AF086627).

EXAMPLE 27

Identification of Rab4/Alpha-catenin-like Protein Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 1-214 of Rab4 (SP accession no. P20338) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 1573-1987 of alpha-catenin-like protein (GB accession no. U97067).

EXAMPLE 28

Identification of Rab4/Rab2 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 1-214 of Rab4 (SP accession no. P20338) as bait was performed. One clone that was identified by this procedure included amino acids 27-177 of Rab2 (SP accession no. P08886).

EXAMPLE 29

Identification of Glut4/PN7065 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 463-509 of Glut4 (Swiss Protein (SP) accession no. P14672) as bait was performed. One clone that was identified by this procedure included novel protein fragment PN7065. The DNA sequence and the predicted protein sequence for PN7065 are set forth in 75 and 76, respetively.

TABLE 75

Nucleotide Sequence of PN7065 (SEQ ID NO:3)

```
CGCCGGTGGATGCGGGCTGAGCCCTGCTTGCCGGGACCCGCCTGCCCCGCCTTCTCCGCA
CACAGCTACACCTCCAACCTGGGCGACTACGATGAGCAGGCGCTGGGTATCATGCAGACC
CTGGGCGTGGACCGGCAGAGGACGGTGGAGTCACTGCAAAACAGCAGCTATAACCACTTT
GCTGCCATTTATTACCTCCTCCTTGAGCGGCTCAAGGAGTATCGGAATGCCCAGTGCGCC
CGCCCCGGGCCTGCCAGGCAGCCGCGGCCTCGGAGCTCGGACCTCAGTGGTTTGGAGGTG
CCTCAGGAAGGTCTTTCCACCGACCCTTTCCGACCTGCCTTGCTGTGCCCGCAGCCGCAG
ACCTTGGTGCAGTCCGTCCTCCAGGCCGAGATGGACTGTGGGCTCCAGAGCTCGCTGCAG
TGGCCCTTGTTCTTCCCGGTGGATGCCAGCTGCAGCGGAGTGTTCCGGCCCCGGCCCGTG
TCCCCAAGCAGCCTGCTGGACACAGCCATCAGTGAGGAGGCCAGGCAGGGGCCGGGCCTA
GAGGAGGAGCAGGACACGCAGGAGTCCCTGCCCAGCAGCACGGGCCGGGGGCACACCCTG
GCCGAGGTCTCCACCCGCCTCTCCCCACTCACCGCGCCAG
```

TABLE 76

Predicted Protein Sequence of PN7065 (SEQ ID NO:4)

```
RRWMRAEPCLPGPACPAFSAHSYTSNLGDYDEQALGIMQTLGVDRQRTVESLQNSSYNHF
AAIYYLLLERLKEYRNAQCARPGPARQPRPRSSDLSGLEVPQEGLSTDPFRPALLCPQPQ
TLVQSVLQAEMDCGLQSSLQWPLFFPVDASCSGVFRPRPVSPSSLLDTAISEEARQGPGL
EEEQDTQESLPSSTGRGHTLAEVSTRLSPLTAP
```

EXAMPLE 30

Identification of Glut4/PN7386 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 478-509 of Glut4 (SP accession no. P14672) as bait was performed. One clone that was identified by this procedure included novel protein fragment PN7386. The DNA sequence and the predicted protein sequence for PN7386 are set forth in Tables 77 and 78, respectively.

TABLE 77

Nucleotide Sequence of PN7386 (SEQ ID NO:5)

GGCCAATGTTGACGTTTTGGTAGGCTATGCAGACATCCATGGAGACTTACTACCTATAAA
TAATGATGATAATTATCACAAAGCTGTTTCAACGGCCAATCCACTGCTTAGGATATTTAT
ACAAAAGAAGGAAGAAGCAGACTACAGTGCCTTTGGTACAGACACGCTAATAAAGAAGAA
GAATGTTTTAACCAACGTATTGCGTCCTGACAACCATAGAAAAAAGCCACATATAGTCAT
TAGTATGCCCCAAGACTTTAGACCTGTGTCTTCTATTATAGACGTGGATATTCTCCCAGA
AACGCATCGTAGGGTACGTCTTTACAAATACGGCACGGAGAAACCCCTAGGATTCTACAT
CCGGGATGGCTCCAGTGTCAGGGTAACACCACATGGCTTAGAAAAGGTTCCAGGGATCTT
TATATCCAGGCTTGTCCCAGGAGGTCTGGCTCAAAGTACAGGACTATTAGCTGTTAATGA
TGAAGTTTTAGAAGTTAATGGCATAGAAGTTTCAGGGAAGAGCCTTGATCAAGTAACAGA
CATGATGATTGCAAATAGCCGTAACCTCATCATAACAGTGAGACCGGCAAACCAGAGGAA
TAATGTTGTGAGGAACAGTCGGACTTCTGGCAGTTCCGGTCAGTCTACTGATAACAGCCT
TCTTGGCTACCCACAGCAGATTGAACCAAGCTTTGAGCCAGAGGATGAAGACAGCGAAGA
AGATGACATTATCATTGAAGACAATGGAGTGCCACAGCAGATTCCAAAAGCTGTTCCTAA
TACTGAGAGCCTGGAGTCATTAACACAGATAGAGCTAAGCTTTGAGTCTGGACAGAATGG
CTTTATTCCCTCTAATGAAGTGAGCTTAGCAGCCATAGCAAGCAGCTCAAACACGGAATT
TGAAACACATGCTCCAGATCAAAAACTCTTAGAAGAAGATGGAACAATCATAACATTATG
AAACCGTGGTTTGAATGTTTTCAGAGTGAGGATGCCATGAGGACTTGTACATTTGGCTAG
TTTAGGCCAATGTTGACGTTTTGGTAGGCTATGCAGACATCCATG

TABLE 78

Predicted Amino Acid Sequence of PN7386 (SEQ ID NO:6)

ANVDVLVGYADIHGDLLPINNDDNYHKAVSTANPLLRIFIQKKEEADYSAFGTDTLIKKK
NVLTNVLRPDNHRKKPHIVISMPQDFRPVSSIIDVDILPETHRRVRLYKYGTEKPLGFYI
RDGSSVRVTPHGLEKVPGIFISRLVPGGLAQSTGLLAVNDEVLEVNGIEVSGKSLDQVTD
MMIANSRNLIITVRPANQRNNVVRNSRTSGSSGQSTDNSLLGYPQQIEPSFEPEDEDSEE
DDIIIEDNGVPQQIPKAVPNTESLESLTQIELSFESGQNGFIPSNEVSLAAIASSSNTEF
ETHAPDQKLLEEDGTIITL

EXAMPLE 31

Identification of OGTase/PN6931 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1618 of OGTase (GenBank (GB) accession no. U77413) as bait was performed. One clone that was identified by this procedure included novel protein fragment PN6931. The DNA sequence and the predicted protein sequence for PN6931 are set forth in Tables 79 and 80, respectively.

TABLE 79

Nucleotide Sequence of PN6931 (SEQ ID NO:7)

AGGGAGGAGAAGCTGAGCCAGGATGAGATCGTGCTGGGCACCAAGGCTGTCATCCAGGGA

CTGGAGACTCTGCGTGGGGAGCATCGTGCCCTGCTGGCTCCTCTGGTTGCACCTGAGGCC
GGCGAAGCCGAGCCTGGCTCGCAGGAGCGCTGCATCCTCCTGCGTCGCTCCCTGGAAGCC
ATTGAGCTTGGGCTGGGGGAGGCCCAGGTGATCTTGGCATTGTCGAGCCACCTGGGGGCT
GTAGAATCAGAGAAGCAGAAGCTGCGGGCGCAGGTGCGGCGTCTGGTGCAGGAGAACCAG
TGGCTGCGTGAGGAGCTGCCGGGGACACAGCAKAAGCTGCAGCGCAGTGAGCAGGCCGTG
GCCCAGCTCGAGGAGGAGAAGCAGCACTTGCTGTTCATGARCCAGATCCGCAGTTGGATG
AAGACGCCTYCCCTAACGAGGAGAAGGGGGACGTCCCCAAAGACACACTGGATGACCTGT
TCCCCAATGAGGATGAGCAGAGCCCAGCCCCTAGCCCAGGAGGAGGGGATGTGTCTGGTC
AGCATGGGGGATACGAGATCCCGGCCCGGCTCCGCACCCTGCACAACTGGTGATCCAATA
CGCCTCACAGGGCCGCTACGAGGTAGCTGTGCCACTCTGCAAGCAGGCACTCGAAGACTG
GAGAAGACGTCAGGCCACGACCACCCTGACGTTGCCACCATGCTGAACATCCTGGCACTG
GTCTATCGGGATCAGAACAAGTACAAGGAGGCTGCCCACCTGCTCAATGATGCTCTGGCC
ATCCGGGAGAAAACACTGGGCAAGGACCACCCAGCCGTGGCTGCGACACTAAACAACCTG
GCAGTCCTGTATAGCGCAGAG

TABLE 80

Predicted Protein Sequence of PN6931 (SEQ ID NO:8)

REEKLSQDEIVLGTKAVIQGLETLRGEHRALLAPLVAPEAGEAEPGSQERCILLRRSLEA
IELGLGEAQVILALSSHLGAVESEKQKLRAQVRRLVQENQWLREELPGTQXKLQRSEQAV
AQLEEEKQHLLFMXQIRSWMKTPXLTRRRGTSPKTHWMTCSPMRMSRAQPLAQEEGMCLV
SMGDTRSRPGSAPCTTGDPIRLTGPLRGSCATLQAGTRRLEKTSGHDHPDVATMLNILAL
VYRDQNKYKEAAHLLNDALAIREKTLGKDHPAVAATLNNLAVLYSAE

EXAMPLE 32

Identification of Naf1b/PN7582 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 233-1568 of Naf1b (GB accession no. AJ011896) as bait was performed. One clone that was identified by this procedure included novel protein fragment PN7582. The DNA sequence and the predicted protein sequence for PN7582 are set forth in Tables 81 and 82, respectively.

TABLE 81

Nucleotide Sequence for PN7582 (SEQ ID NO:9)

ATTTCAAGGGGGCTGCTGTACCCCCAGGCATGTGTCTGTATATCGCACAGGAAGAAGGAA
AGTAAGGACATTGCCAGCAAATATCTTACATCTCATCAGCCTATACTGTGTCTCCTGACC
ACTCCTAACTGCAAAGGATGCTGGGAAAAAAAGAGCATTGTAGCTTTTCCAGCCTCTGTG
GTAGGCGCAGATAAGGGATTAGAGTTGGGTGTTACTGAATCAATGTATCAGACACTTCTC
AGTCAGGCTAGAGCCAGATTTAACTAGATTTAGCAGGAAAAGTATGTTTCTTTCACCTGC
ATGTAATGAAGGAAATCTATGTCCTTCATACACTTAATAAACCTGTAAGTCTCTACTATG
GGCAGGTACTGTGCTAGCTAGACATTACAATGTGTGGGGGCAGACACAAAGATGGGAACA
GTAGACACTGGGGAACCCTAGAGGGGGGAGGTTGGGAGTAGGGGAAGGGTTGAAAAATGA
TTGGGTACTATGCTCACTACCTGGGTGATGGGATCATTTGTACACCAAATGCCAGCAACA
CACAATTTACCCGTGTAACACACCGGCACGTGTACCCCCTGAACCTAAGATGAAAGCCGA
A

TABLE 82

Predicted Amino Acid Sequence for PN7582 (SEQ ID NO:10)

ISRGLLYPQACVCISHRKKESKDIASKYLTSHQPILCLLTTPNCKGCWEKKSIVAFPASV
VGADKGLELGVTESMYQTLLSQARARFN

EXAMPLE 33

Identification of OGTase/Talin Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (Genbank (GB) accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids 812 et seq. of talin (GB accession no. AF078828).

EXAMPLE 34

Identification of OGTase/MOP2 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (Genbank (GB) accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids 397-546 of MOP2 (Swiss Protein (SP) accession no. Q99814).

EXAMPLE 35

Identification of OGTase/Clone 25100 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (Genbank (GB) accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 31-1014 of clone 25100 (GB accession no. AF121780).

EXAMPLE 36

Identification of OGTase/KIAA0443 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (Genbank (GB) accession no. U77413) as bait was performed.

One clone that was identified by this procedure included amino acids encoded by nucleotides 3468-4106 of KIAA0443 (GB accession no. AB007903).

EXAMPLE 37

Identification of OGTase/EGR1 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (Genbank (GB) accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids 415-544 of EGR1 (SP accession no. P18146).

EXAMPLE 38

Identification of OGTase/Dynamin II Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (Genbank (GB) accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids 515-823 of dynamin II (SP accession no. P50570).

EXAMPLE 39

Identification of OGTase/INT-6 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (Genbank (GB) accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 824-1191 of Int-6 (GB accession no. U62962).

EXAMPLE 40

Identification of OGTase/HSPC028 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (Genbank (GB) accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 58-443 of HSPC028 (GB accession no. AF083246).

EXAMPLE 41

Identification of OGTase/BAP31 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (Genbank (GB) accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids 116-215 of BAP31 (SP accession no. P51572).

EXAMPLE 42

Identification of OGTase/Interferon-Ind Protein Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1349 of OGTase (Genbank (GB) accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids 1 et seq. of interferon-ind protein (SP accession no. P09913).

EXAMPLE 43

Identification of Glut4/Beta-catenin Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 463-509 of Glut4 (Swiss Protein (SP) accession no. P14672) as bait was performed. One clone that was identified by this procedure included amino acids 579-782 of Beta-catenin (SP accession no. P35222).

EXAMPLE 44

Identification of Glut4/Alpha-SNAP Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 463-509 of Glut4 (SP accession no. P14672) as bait was performed. One clone that was identified by this procedure included amino acids 36-241 of Alpha-SNAP (SP accession no. P54920).

EXAMPLE 45

Identification of Glut4/MAPKKK6 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 463-509 of Glut4 (SP accession no. P14672) as bait was performed. One clone that was identified by this procedure included amino acids 824-1012 of MAPKKK6 (GenBank (GB) accession no. AF100318).

EXAMPLE 46

Identification of GLUT4/Tropomyosin 3 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 434-509 of Glut4 (SP accession no. P14672) as bait was performed. One clone that was identified by this procedure included amino acids 171-286 of tropomyosin 3 (SP accession no. P06753).

EXAMPLE 47

Identification of GLUT1/DRAL/FHL2 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 448-492 of Glut1 (SP accession no. P11166) as bait was performed. One clone that was identified by this procedure included amino acids 1-280 of DRAL/FHL2 (SP accession no. Q13229).

EXAMPLE 48

Identification of GLUT1/MYSA Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 448-492 of Glut1 (SP accession no. P11166) as bait was performed. One clone that was identified by this procedure included amino acids 1589-1909 of MYSA (SP accession no. P13533).

EXAMPLE 49

Identification of IRAP/SG2NA Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 2311-3136 of IRAP (GB accession no. U62768) as bait was performed. One clone that was identified by this procedure included amino acids 623-713 of SG2NA (SP accession no. P70483).

EXAMPLE 50

Identification of IRAP/SLAP-2 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 62-880 of IRAP (GB accession no. U62768) as bait was performed. One clone that was identified by this procedure included amino acids 236-453 of SLAP-2 (GB accession no. AF100750).

EXAMPLE 51

Identification of OGTase/14-3-3 Epsilon Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1618 of OGTase (GB accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids 21-232 of 14-3-3 epsilon (SP accession no. P29360).

EXAMPLE 52

Identification of PI-3K85/Chromogranin Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 1-250 of PI-3 kinase p85 subunit (SP accession no. P27986) as bait was performed. One clone that was identified by this procedure included amino acids 2-322 of chromogranin (SP accession no. P13521).

EXAMPLE 53

Identification of PI-3K85/SLP-76 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 320-440 of PI-3 kinase p85 subunit (SP accession no. P27986) as bait was performed. One clone that was identified by this procedure included amino acids 291-486 of SLP-76 (GB accession no. U20158).

EXAMPLE 54

Identification of PI-3K85/SLP-76 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 600-724 of PI-3 kinase p85 subunit (SP accession no. P27986) as bait was performed. One clone that was identified by this procedure included amino acids 291-486 of SLP-76 (GB accession no. U20158).

EXAMPLE 55

Identification of PI-3K85/14-3-3-zeta Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 425-630 of PI-3 kinase p85 subunit (SP accession no. P27986) as bait was performed. One clone that was identified by this procedure included amino acids 79-246 of 14-3-3-zeta (AP accession no. P29213).

EXAMPLE 56

Identification of PI-3K85/14-3-3-eta Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 425-630 of PI-3 kinase p85 subunit (SP accession no. P27986) as bait was performed. One clone that was identified by this procedure included amino acids beginning with residue 91 of 14-3-3-eta (SP accession no. Q04917).

EXAMPLE 57

Identification of PI-3K85/TACC2 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 600-724 of PI-3 kinase p85 subunit (SP accession no. P27986) as bait was performed. One clone that was identified by this procedure included amino acids 350-600 of TACC2 (GB accession no. AF095791).

EXAMPLE 58

Identification of Glut4/MM-1 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 463-509 of Glut4 (Swiss Protein (SP) accession no. P14672) as bait was performed. One clone that was identified by this procedure included amino acids 27-168 of MM-1 (GenBank (GB) accession no. D89667).

EXAMPLE 59

Identification of Glut1/KIAA0144 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 463-492 of Glut1 (SP accession no. P11166) as bait was performed. One clone that was identified by this procedure included amino acids beginning with residue 691 of KIAA0144 (GB accession no. D63478).

EXAMPLE 60

Identification of Glut1/Dynamin Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 463-492 of Glut1 (Swiss Protein (SP) accession no. P11166) as bait was performed. One clone that was identified by this procedure included amino acids 104-365 of dynamin (GB accession no. L07807).

EXAMPLE 61

Identification of Glut 1/Clone 25204 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 463-492 of Glut1 (Swiss Protein (SP) accession no. P11166) as bait was performed. One clone that was identified by this procedure included undetermined amino acids residues of Clone 25204 (GB accession no. AF131749).

EXAMPLE 62

Identification of IRAP/VAP-A Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 62-880 of IRAP (GB accession no. U62768) as bait was performed. One clone that was identified by this procedure included amino acids 3-243 of VAP-A (GB accession no. AF086627).

EXAMPLE 63

Identification of OGTase/Nafl a Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 1016-1618 of OGTase (GB accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids 41-486 of Nafl a (GB accession no. AJ011895).

EXAMPLE 64

Identification of OGTasePP5/Alpha-2-Catenin

A yeast two-hybrid system as described in Example 1 using acids encoded by nucleotides 1016-1618 of OGTase (GB accession no. U77413) as bait was performed. One clone that was identified by this procedure included amino acids 366-469 of Alpha-2-Catenin (GB accession no. M94151).

EXAMPLE 65

Identification of PI-3K110/TRIP15 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 1-300 of PI-3K110 (SP accession no. P42338) as bait was performed. One clone that was identified by this procedure included amino acids 24-233 of TRIP15 (GB accession no. L40388).

EXAMPLE 66

Identification of Glut4/14-3-3 Zeta Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 463-509 of Glut4 (Swiss Protein (SP) accession no. P14672) as bait was performed. Three clones that were identified by this procedure included amino acids 1-121, 1-200 and 1-209 of 14-3-3 zeta (SP accession no. P29213).

EXAMPLE 67

Identification of Glut4/KIAA0282 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 463-509 of Glut4 (Swiss Protein (SP) accession no. P14672) as bait was performed. One clone that was identified by this procedure included amino acids 229-379 of KIAA0282 (GenBank (GB) accession no. D87458), an efp-like protein.

EXAMPLE 68

Identification of Glut4/Tankyrase Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 463-509 of Glut4 (Swiss Protein (SP) accession no. P14672) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 1110-1750 of tankyrase (GB accession no. AF082557).

EXAMPLE 69

Identification of IRAP/PTPZ Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 62-880 of IRAP (GB accession no. U62768) as bait was performed. One clone that was identified by this procedure included amino acids 1420-1780 of PTPZ (SP accession no. P23471).

EXAMPLE 70

Identification of IRAP/βSpectrin Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 2311-3136 of IRAP (GB accession no. U62768) as bait was performed. One clone that

was identified by this procedure included amino acids 1487-1993 of β-spectrin (SP accession no. Q01082).

EXAMPLE 71

Identification of IRAP/PI-3K85 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids encoded by nucleotides 2311-3136 of IRAP (GB accession no. U62768) as bait was performed. One clone that was identified by this procedure included amino acids 498-544 of PI-3 kinase p85 subunit (SP accession no. P27986).

EXAMPLE 72

Identification of PP5/HSP89 Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 1-150 of PP5 (SP accession no. P53041) as bait was performed. One clone that was identified by this procedure included amino acids 517-681 of Hsp89 (SP accession no. P07900).

EXAMPLE 73

Identification of PP5/Tankyrase Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 1-150 of PP5 (SP accession no. P53041) as bait was performed. One clone that was identified by this procedure included amino acids encoded by nucleotides 1110-1750 and nucleotides 1809-2257 of tankyrase (GB accession no. AF082557).

EXAMPLE 74

Identification of PI-3K85/Tankyrase Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 320-440 of PI-3 kinase p85 subunit (SP accession no. P27986) as bait was performed. Two clones that were identified by this procedure included amino acids encoded by nucleotides 1110-1750 of tankyrase (GB accession no. AF082557).

EXAMPLE 75

Identification of PI-3K110/APP Interaction

A yeast two-hybrid system as described in Example 1 using amino acids 1-300 of PI-3 kinase p110 subunit (SP accession no. P42338) as bait was performed. One clone that was identified by this procedure included amino acids 374-546 of APP (SP accession no. P05067).

EXAMPLE 76

Generation of Polyclonal Antibody Against Protein Complexes

As shown above, Glut4 interacts with CARP to form a complex. A complex of the two proteins is prepared, e.g., by mixing purified preparations of each of the two proteins. If desired, the protein complex can be stabilized by cross-linking the proteins in the complex, by methods known to those of skill in the art. The protein complex is used to immunize rabbits and mice using a procedure similar to that described by Harlow et al. (1988). This procedure has been shown to generate Abs against various other proteins (for example, see Kraemer et al., 1993).

Briefly, purified protein complex is used as immunogen in rabbits. Rabbits are immunized with 100 μg of the protein in complete Freund's adjuvant and boosted twice in three-week intervals, first with 100 μg of immunogen in incomplete Freund's adjuvant, and followed by 100 μg of immunogen in PBS. Antibody-containing serum is collected two weeks thereafter. The antisera is preadsorbed with Glut4 and CARP, such that the remaining antisera comprises antibodies which bind conformational epitopes, i.e., complex-specific epitopes, present on the Glut4-CARP complex but not on the monomers.

Polyclonal antibodies against each of the complexes set forth in Tables 1-73 are prepared in a similar manner by mixing the specified proteins together, immunizing an animal and isolating antibodies specific for the protein complex, but not for the individual proteins.

EXAMPLE 77

Generation of Monoclonal Antibodies Specific for Protein Complexes

Monoclonal antibodies are generated according to the following protocol. Mice are immunized with immunogen comprising Glut4/CARP complexes conjugated to keyhole limpet hemocyanin using glutaraldehyde or EDC as is well known in the art. The complexes can be prepared as described in Example 76, and may also be stabilized by cross-linking. The immunogen is mixed with an adjuvant. Each mouse receives four injections of 10 to 100 μg of immunogen, and after the fourth injection blood samples are taken from the mice to determine if the serum contains antibody to the immunogen. Serum titer is determined by ELISA or RIA. Mice with sera indicating the presence of antibody to the immunogen are selected for hybridoma production.

Spleens are removed from immune mice and a single-cell suspension is prepared (Harlow et al., 1988). Cell fusions are performed essentially as described by Kohler et al. (1975). Briefly, P3.65.3 myeloma cells (American Type Culture Collection, Rockville, MD) or NS-1 myeloma cells are fused with immune spleen cells using polyethylene glycol as described by Harlow et al. (1988). Cells are plated at a density of $2x10^5$ cells/well in 96-well tissue culture plates. Individual wells are examined for growth, and the supernatants of wells with growth are tested for the presence of Glut4/CARP complex-specific antibodies by ELISA or RIA using Glut4/CARP complex as target protein. Cells in positive wells are expanded and subcloned to establish and confirm monoclonality.

Clones with the desired specificities are expanded and grown as ascites in mice or in a hollow fiber system to produce sufficient quantities of antibodies for characterization and assay development. Antibodies are tested for binding to Glut4 alone or to CARP alone, to determine which are specific for the Glut4/CARP complex as opposed to those that bind to the individual proteins.

Monoclonal antibodies against each of the complexes set forth in Tables 1-73 are prepared in a similar manner by mixing the specified proteins together, immunizing an animal, fusing spleen cells with myeloma cells and isolating clones which produce antibodies specific for the protein complex, but not for the individual proteins.

EXAMPLE 78

In vitro Identification of Modulators for Protein-Protein Interactions

The present invention is useful in screening for agents that modulate the interaction of Glut4 and CARP. The knowledge that Glut4 and CARP form a complex is useful in designing such

assays. Candidate agents are screened by mixing Glut4 and CARP (a) in the presence of a candidate agent, and (b) in the absence of the candidate agent. The amount of complex formed is measured for each sample. An agent modulates the interaction of Glut4 and CARP if the amount of complex formed in the presence of the agent is greater than (promoting the interaction), or less than (inhibiting the interaction) the amount of complex formed in the absence of the agent. The amount of complex is measured by a binding assay, which shows the formation of the complex, or by using antibodies immunoreactive to the complex.

Briefly, a binding assay is performed in which immobilized Glut4 is used to bind labeled CARP. The labeled CARP is contacted with the immobilized Glut4 under aqueous conditions that permit specific binding of the two proteins to form an Glut4/CARP complex in the absence of an added test agent. Particular aqueous conditions may be selected according to conventional methods. Any reaction condition can be used as long as specific binding of Glut4/CARP occurs in the control reaction. A parallel binding assay is performed in which the test agent is added to the reaction mixture. The amount of labeled CARP bound to the immobilized Glut4 is determined for the reactions in the absence or presence of the test agent. If the amount of bound, labeled CARP in the presence of the test agent is different than the amount of bound labeled CARP in the absence of the test agent, the test agent is a modulator of the interaction of Glut4 and CARP.

Candidate agents for modulating the interaction of each of the protein complexes set forth in Tables 1-73 are screened *in vitro* in a similar manner.

EXAMPLE 79

In vivo Identification of Modulators for Protein-Protein Interactions

In addition to the *in vitro* method described in Example 78, an *in vivo* assay can also be used to screen for agents which modulate the interaction of Glut4 and CARP. Briefly, a yeast two-hybrid system is used in which the yeast cells express (1) a first fusion protein comprising Glut4 or a fragment thereof and a first transcriptional regulatory protein sequence, e.g., GAL4 activation domain, (2) a second fusion protein comprising CARP or a fragment thereof and a second transcriptional regulatory protein sequence, e.g., GAL4 DNA-binding domain, and (3) a reporter gene, e.g., β-galactosidase, which is transcribed when an intermolecular complex comprising the first fusion protein and the second fusion protein is formed. Parallel reactions are performed in the absence of a test agent as the control and in the presence of the test agent. A functional Glut4/CARP complex is detected by detecting the amount of reporter gene expressed. If the amount of reporter

gene expression in the presence of the test agent is different than the amount of reporter gene expression in the absence of the test agent, the test agent is a modulator of the interaction of Glut4 and CARP.

Candidate agents for modulating the interaction of each of the protein complexes set forth in Tables 1-73 are screened *in vivo* in a similar manner.

While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

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U.S. Patent No. 5,773,218

WHAT IS CLAIMED IS:

1. An isolated protein complex comprising two proteins, the protein complex selected from the group consisting of

(a) a complex set forth in Table 1;

(b) a complex set forth in Table 2;

(c) a complex set forth in Table 3;

(d) a complex set forth in Table 4;

(e) a complex set forth in Table 5;

(f) a complex set forth in Table 6;

(g) a complex set forth in Table 7;

(h) a complex set forth in Table 8;

(i) a complex set forth in Table 9;

(j) a complex set forth in Table 10;

(k) a complex set forth in Table 11;

(l) a complex set forth in Table 12;

(m) a complex set forth in Table 13;

(n) a complex set forth in Table 14;.

(o) a complex set forth in Table 15;

(p) a complex set forth in Table 16;

(q) a complex set forth in Table 17;

(r) a complex set forth in Table 18;

(s) a complex set forth in Table 19;

(t) a complex set forth in Table 20;

(u) a complex set forth in Table 21;

(v) a complex set forth in Table 22;

(w) a complex set forth in Table 23;

(x) a complex set forth in Table 24;

(y) a complex set forth in Table 25;

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(aa) a complex set forth in Table 27;

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(bf) a complex set forth in Table 58;

(bg) a complex set forth in Table 59;

(bh) a complex set forth in Table 60;

(bi) a complex set forth in Table 61;

(bj) a complex set forth in Table 62;

(bk) a complex set forth in Table 63;

(bl) a complex set forth in Table 64;

(bm) a complex set forth in Table 65;

(bn) a complex set forth in Table 66;

(bo) a complex set forth in Table 67;

(bp) a complex set forth in Table 68;.

(bq) a complex set forth in Table 69;

(br) a complex set forth in Table 70;

(bs) a complex set forth in Table 71;

(bt) a complex set forth in Table 72; and

(bu) a complex set forth in Table 73.

2. The protein complex of claim 1, wherein said protein complex comprises complete proteins.

3. The protein complex of claim 1, wherein said protein complex comprises a fragment of one protein and a complete protein of anther protein.

4. The protein complex of claim 1, wherein said protein complex comprises fragments of proteins.

5. An isolated antibody selectively immunoreactive with the protein complex of claim 1.

6. The antibody of claim 5, wherein said antibody is a monoclonal antibody.

7. A method for diagnosing a physiological disorder in an animal, which comprises assaying for:

(a) whether a protein complex set forth in any one of Tables 1-73 is present in a tissue extract;

(b) the ability of proteins to form a protein complex set forth in any one of Tables 1-73; and

(c) a mutation in a gene encoding a protein of a protein complex set forth in any one of Tables 1-73.

8. The method of claim 7, wherein said animal is a human.

9. The method of claim 7, wherein the diagnosis is for a predisposition to said physiological disorder.

10. The method of claim 7, wherein the diagnosis is for the existence of said physiological disorder.

11. The method of claim 7, wherein said assay comprises a yeast two-hybrid assay.

12. The method of claim 7, wherein said assay comprises measuring *in vitro* a complex formed by combining the proteins of the protein complex, said proteins isolated from said animal.

13. The method of claim 12, wherein said complex is measured by binding with an antibody specific for said complex.

14. The method of claim 7, wherein said assay comprises mixing an antibody specific for said protein complex with a tissue extract from said animal and measuring the binding of said antibody.

15. A method for determining whether a mutation in a gene encoding one of the proteins of a protein complex set forth in any one of Tables 1-73 is useful for diagnosing a physiological disorder, which comprises assaying for the ability of said protein with said mutation to form a complex with the other protein of said protein complex, wherein an inability to form said complex is indicative of said mutation being useful for diagnosing a physiological disorder.

16. The method of claim 15, wherein said gene is an animal gene.

17. The method of claim 16, wherein said animal is a human.

18. The method of claim 15, wherein the diagnosis is for a predisposition to a physiological disorder.

19. The method of claim 15, wherein the diagnosis is for the existence of a physiological disorder.

20. The method of claim 15, wherein said assay comprises a yeast two-hybrid assay.

21. The method of claim 15, wherein said assay comprises measuring *in vitro* a complex formed by combining the proteins of the protein complex, said proteins isolated from an animal.

22. The method of claim 21, wherein said animal is a human.

23. The method of claim 21, wherein said complex is measured by binding with an antibody specific for said complex.

24. A method for screening for drug candidates capable of modulating the interaction of the proteins of a protein complex set forth in any one of Tables 1-73, which comprises:

(a) combining the proteins of said protein complex in the presence of a drug to form a first complex;

(b) combining the proteins in the absence of said drug to form a second complex;

(c) measuring the amount of said first complex and said second complex; and

(d) comparing the amount of said first complex with the amount of said second complex,

wherein if the amount of said first complex is greater than, or less than the amount of said second complex, then the drug is a drug candidate for modulating the interaction of the proteins of said protein complex..

25. The method of claim 24, wherein said screening is an *in vitro* screening.

26. The method of claim 24, wherein said complex is measured by binding with an antibody specific for said protein complexes.

27. The method of claim 24, wherein if the amount of said first complex is greater than the amount of said second complex, then said drug is a drug candidate for promoting the interaction of said proteins.

28. The method of claim 24, wherein if the amount of said first complex is less than the amount of said second complex, then said drug is a drug candidate for inhibiting the interaction of said proteins.

29. A non-human animal model for a physiological disorder wherein the genome of said animal or an ancestor thereof has been modified such that the formation of a protein complex set forth in any one of Tables 1-73 has been altered.

30. The non-human animal model of claim 29, wherein the formation of said protein complex has been altered as a result of:

(a) over-expression of at least one of the proteins of said protein complex;

(b) replacement of a gene for at least one of the proteins of said protein complex with a gene from a second animal and expression of said protein;

(c) expression of a mutant form of at least one of the proteins of said protein complex;

(d) a lack of expression of at least one of the proteins of said protein complex; or

(e) reduced expression of at least one of the proteins of said protein complex.

31. A cell line obtained from the animal model of claim 29.

32. A non-human animal model for a physiological disorder, wherein the biological activity of a protein complex set forth in any one of Tables 1-73 has been altered.

33. The non-human animal model of claim 32, wherein said biological activity has been altered as a result of:

(a) disrupting the formation of said complex; or

(b) disrupting the action of said complex.

34. The non-human animal model of claim 32, wherein the formation of said complex is disrupted by binding an antibody to at least one of the proteins which form said protein complex.

35. The non-human animal model of claim 32, wherein the action of said complex is disrupted by binding an antibody to said complex.

36. The non-human animal model of claim 32, wherein the formation of said complex is disrupted by binding a small molecule to at least one of the proteins which form said protein complex.

37. The non-human animal model of claim 32, wherein the action of said complex is disrupted by binding a small molecule to said complex.

38. A cell in which the genome of cells of said cell line has been modified to produce at least one protein complex set forth in any one of Tables 1-73.

39. A cell line in which the genome of the cells of said cell line has been modified to eliminate at least one protein of a protein complex set forth in any one of Tables 1-73.

40. A method of screening for drug candidates useful in treating a physiological disorder which comprises the steps of:

(a) measuring the activity of a protein selected from the proteins set forth in Tables 1-73 in the presence of a drug,

(b) measuring the activity of said protein in the absence of said drug, and

(c) comparing the activity measured in steps (1) and (2),

wherein if there is a difference in activity, then said drug is a drug candidate for treating said physiological disorder.

41. An isolated nucleic acid encoding a polypeptide comprising an amino acid sequence selected from the group consisting of amino acid sequences set forth in SEQ ID NOs:4, 6, 8 and10.

42. The isolated nucleic acid of claim 41 comprising a nucleotide sequence selected from the group consisting of nucleotide sequences set forth in SEQ ID NOs:3, 5, 7 and 9.

43. An isolated polypeptide comprising an amino acid amino acid sequence selected from the group consisting of amino acid sequences set forth in SEQ ID NOs:4, 6, 8 and10.

SEQUENCE LISTING

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Myriad Genetics, Inc.

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gcc ttc tcc gca cac agc tac acc tcc aac ctg ggc gac tac gat gag   96
Ala Phe Ser Ala His Ser Tyr Thr Ser Asn Leu Gly Asp Tyr Asp Glu
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cag gcg ctg ggt atc atg cag acc ctg ggc gtg gac cgg cag agg acg   144
Gln Ala Leu Gly Ile Met Gln Thr Leu Gly Val Asp Arg Gln Arg Thr
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gtg gag tca ctg caa aac agc agc tat aac cac ttt gct gcc att tat   192
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65                  70                  75                  80

cgc ccc ggg cct gcc agg cag ccg cgg cct cgg agc tcg gac ctc agt   288
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ggt ttg gag gtg cct cag gaa ggt ctt tcc acc gac cct ttc cga cct   336
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Ala Leu Leu Cys Pro Gln Pro Gln Thr Leu Val Gln Ser Val Leu Gln
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     50                  55                  60

Tyr Leu Leu Leu Glu Arg Leu Lys Glu Tyr Arg Asn Ala Gln Cys Ala
 65                  70                  75                  80

Arg Pro Gly Pro Ala Arg Gln Pro Arg Pro Arg Ser Ser Asp Leu Ser
                 85                  90                  95

Gly Leu Glu Val Pro Gln Glu Gly Leu Ser Thr Asp Pro Phe Arg Pro
            100                 105                 110

Ala Leu Leu Cys Pro Gln Pro Gln Thr Leu Val Gln Ser Val Leu Gln
        115                 120                 125

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cta cct ata aat aat gat gat aat tat cac aaa gct gtt tca acg gcc   97
Leu Pro Ile Asn Asn Asp Asp Asn Tyr His Lys Ala Val Ser Thr Ala
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aat cca ctg ctt agg ata ttt ata caa aag aag gaa gaa gca gac tac   145
Asn Pro Leu Leu Arg Ile Phe Ile Gln Lys Lys Glu Glu Ala Asp Tyr
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agt gcc ttt ggt aca gac acg cta ata aag aag aag aat gtt tta acc   193
Ser Ala Phe Gly Thr Asp Thr Leu Ile Lys Lys Lys Asn Val Leu Thr
     50                  55                  60

aac gta ttg cgt cct gac aac cat aga aaa aag cca cat ata gtc att   241
Asn Val Leu Arg Pro Asp Asn His Arg Lys Lys Pro His Ile Val Ile
 65                  70                  75                  80

agt atg ccc caa gac ttt aga cct gtg tct tct att ata gac gtg gat   289
Ser Met Pro Gln Asp Phe Arg Pro Val Ser Ser Ile Ile Asp Val Asp
                 85                  90                  95

att ctc cca gaa acg cat cgt agg gta cgt ctt tac aaa tac ggc acg   337
Ile Leu Pro Glu Thr His Arg Arg Val Arg Leu Tyr Lys Tyr Gly Thr
            100                 105                 110

gag aaa ccc cta gga ttc tac atc cgg gat ggc tcc agt gtc agg gta   385
Glu Lys Pro Leu Gly Phe Tyr Ile Arg Asp Gly Ser Ser Val Arg Val
        115                 120                 125

aca cca cat ggc tta gaa aag gtt cca ggg atc ttt ata tcc agg ctt   433
Thr Pro His Gly Leu Glu Lys Val Pro Gly Ile Phe Ile Ser Arg Leu
    130                 135                 140

gtc cca gga ggt ctg gct caa agt aca gga cta tta gct gtt aat gat   481
Val Pro Gly Gly Leu Ala Gln Ser Thr Gly Leu Leu Ala Val Asn Asp
145                 150                 155                 160

gaa gtt tta gaa gtt aat ggc ata gaa gtt tca ggg aag agc ctt gat   529
Glu Val Leu Glu Val Asn Gly Ile Glu Val Ser Gly Lys Ser Leu Asp
                165                 170                 175

caa gta aca gac atg atg att gca aat agc cgt aac ctc atc ata aca   577
Gln Val Thr Asp Met Met Ile Ala Asn Ser Arg Asn Leu Ile Ile Thr
            180                 185                 190

gtg aga ccg gca aac cag agg aat aat gtt gtg agg aac agt cgg act   625
Val Arg Pro Ala Asn Gln Arg Asn Asn Val Val Arg Asn Ser Arg Thr
        195                 200                 205

tct ggc agt tcc ggt cag tct act gat aac agc ctt ctt ggc tac cca   673
Ser Gly Ser Ser Gly Gln Ser Thr Asp Asn Ser Leu Leu Gly Tyr Pro
    210                 215                 220

cag cag att gaa cca agc ttt gag cca gag gat gaa gac agc gaa gaa   721
Gln Gln Ile Glu Pro Ser Phe Glu Pro Glu Asp Glu Asp Ser Glu Glu
225                 230                 235                 240

gat gac att atc att gaa gac aat gga gtg cca cag cag att cca aaa   769
Asp Asp Ile Ile Ile Glu Asp Asn Gly Val Pro Gln Gln Ile Pro Lys
                245                 250                 255

gct gtt cct aat act gag agc ctg gag tca tta aca cag ata gag cta   817
Ala Val Pro Asn Thr Glu Ser Leu Glu Ser Leu Thr Gln Ile Glu Leu
            260                 265                 270

agc ttt gag tct gga cag aat ggc ttt att ccc tct aat gaa gtg agc   865
Ser Phe Glu Ser Gly Gln Asn Gly Phe Ile Pro Ser Asn Glu Val Ser
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tta gca gcc ata gca agc agc tca aac acg gaa ttt gaa aca cat gct   913
Leu Ala Ala Ile Ala Ser Ser Ser Asn Thr Glu Phe Glu Thr His Ala
    290                 295                 300

cca gat caa aaa ctc tta gaa gaa gat gga aca atc ata aca tta       958
Pro Asp Gln Lys Leu Leu Glu Glu Asp Gly Thr Ile Ile Thr Leu
305                 310                 315

tgaaaccgtg gtttgaatgt tttcagagtg aggatgccat gaggacttgt acatttggct 1018

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Asn Pro Leu Leu Arg Ile Phe Ile Gln Lys Lys Glu Glu Ala Asp Tyr
        35                  40                  45

Ser Ala Phe Gly Thr Asp Thr Leu Ile Lys Lys Lys Asn Val Leu Thr
    50                  55                  60

Asn Val Leu Arg Pro Asp Asn His Arg Lys Lys Pro His Ile Val Ile
65                  70                  75                  80

Ser Met Pro Gln Asp Phe Arg Pro Val Ser Ser Ile Ile Asp Val Asp
                85                  90                  95

Ile Leu Pro Glu Thr His Arg Arg Val Arg Leu Tyr Lys Tyr Gly Thr
            100                 105                 110

Glu Lys Pro Leu Gly Phe Tyr Ile Arg Asp Gly Ser Ser Val Arg Val
        115                 120                 125

Thr Pro His Gly Leu Glu Lys Val Pro Gly Ile Phe Ile Ser Arg Leu
    130                 135                 140

Val Pro Gly Gly Leu Ala Gln Ser Thr Gly Leu Leu Ala Val Asn Asp
145                 150                 155                 160

Glu Val Leu Glu Val Asn Gly Ile Glu Val Ser Gly Lys Ser Leu Asp
                165                 170                 175

Gln Val Thr Asp Met Met Ile Ala Asn Ser Arg Asn Leu Ile Ile Thr
            180                 185                 190

Val Arg Pro Ala Asn Gln Arg Asn Asn Val Val Arg Asn Ser Arg Thr
        195                 200                 205

Ser Gly Ser Ser Gly Gln Ser Thr Asp Asn Ser Leu Leu Gly Tyr Pro
    210                 215                 220

Gln Gln Ile Glu Pro Ser Phe Glu Pro Glu Asp Glu Asp Ser Glu Glu
225                 230                 235                 240
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Asp Asp Ile Ile Ile Glu Asp Asn Gly Val Pro Gln Gln Ile Pro Lys
                245                 250                 255

Ala Val Pro Asn Thr Glu Ser Leu Glu Ser Leu Thr Gln Ile Glu Leu
            260                 265                 270

Ser Phe Glu Ser Gly Gln Asn Gly Phe Ile Pro Ser Asn Glu Val Ser
        275                 280                 285

Leu Ala Ala Ile Ala Ser Ser Ser Asn Thr Glu Phe Glu Thr His Ala
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Pro Asp Gln Lys Leu Leu Glu Glu Asp Gly Thr Ile Ile Thr Leu
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gtc atc cag gga ctg gag act ctg cgt ggg gag cat cgt gcc ctg ctg   96
Val Ile Gln Gly Leu Glu Thr Leu Arg Gly Glu His Arg Ala Leu Leu
             20                  25                  30

gct cct ctg gtt gca cct gag gcc ggc gaa gcc gag cct ggc tcg cag   144
Ala Pro Leu Val Ala Pro Glu Ala Gly Glu Ala Glu Pro Gly Ser Gln
         35                  40                  45

gag cgc tgc atc ctc ctg cgt cgc tcc ctg gaa gcc att gag ctt ggg   192
Glu Arg Cys Ile Leu Leu Arg Arg Ser Leu Glu Ala Ile Glu Leu Gly
     50                  55                  60

ctg ggg gag gcc cag gtg atc ttg gca ttg tcg agc cac ctg ggg gct   240
Leu Gly Glu Ala Gln Val Ile Leu Ala Leu Ser Ser His Leu Gly Ala
 65                  70                  75                  80

gta gaa tca gag aag cag aag ctg cgg gcg cag gtg cgg cgt ctg gtg   288
Val Glu Ser Glu Lys Gln Lys Leu Arg Ala Gln Val Arg Arg Leu Val
                 85                  90                  95

cag gag aac cag tgg ctg cgt gag gag ctg ccg ggg aca cag cak aag   336
Gln Glu Asn Gln Trp Leu Arg Glu Glu Leu Pro Gly Thr Gln Xaa Lys
            100                 105                 110

ctg cag cgc agt gag cag gcc gtg gcc cag ctc gag gag gag aag cag   384
Leu Gln Arg Ser Glu Gln Ala Val Ala Gln Leu Glu Glu Glu Lys Gln
        115                 120                 125

cac ttg ctg ttc atg arc cag atc cgc agt tgg atg aag acg cct ycc   432
His Leu Leu Phe Met Xaa Gln Ile Arg Ser Trp Met Lys Thr Pro Xaa
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cta acg agg aga agg ggg acg tcc cca aag aca cac tgg atg acc tgt   480
Leu Thr Arg Arg Arg Gly Thr Ser Pro Lys Thr His Trp Met Thr Cys
145                 150                 155                 160
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tcc cca atg agg atg agc aga gcc cag ccc cta gcc cag gag gag ggg   528
Ser Pro Met Arg Met Ser Arg Ala Gln Pro Leu Ala Gln Glu Glu Gly
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atg tgt ctg gtc agc atg ggg gat acg aga tcc cgg ccc ggc tcc gca   576
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            180                 185                 190

ccc tgc aca act ggt gat cca ata cgc ctc aca ggg ccg cta cga ggt   624
Pro Cys Thr Thr Gly Asp Pro Ile Arg Leu Thr Gly Pro Leu Arg Gly
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agc tgt gcc act ctg caa gca ggc act cga aga ctg gag aag acg tca   672
Ser Cys Ala Thr Leu Gln Ala Gly Thr Arg Arg Leu Glu Lys Thr Ser
    210                 215                 220

ggc cac gac cac cct gac gtt gcc acc atg ctg aac atc ctg gca ctg   720
Gly His Asp His Pro Asp Val Ala Thr Met Leu Asn Ile Leu Ala Leu
225                 230                 235                 240

gtc tat cgg gat cag aac aag tac aag gag gct gcc cac ctg ctc aat   768
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                245                 250                 255

gat gct ctg gcc atc cgg gag aaa aca ctg ggc aag gac cac cca gcc   816
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            260                 265                 270

gtg gct gcg aca cta aac aac ctg gca gtc ctg tat agc gca gag       861
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Ala Pro Leu Val Ala Pro Glu Ala Gly Glu Ala Glu Pro Gly Ser Gln
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Glu Arg Cys Ile Leu Leu Arg Arg Ser Leu Glu Ala Ile Glu Leu Gly
    50                  55                  60

Leu Gly Glu Ala Gln Val Ile Leu Ala Leu Ser Ser His Leu Gly Ala
65                  70                  75                  80

Val Glu Ser Glu Lys Gln Lys Leu Arg Ala Gln Val Arg Arg Leu Val
                85                  90                  95

Gln Glu Asn Gln Trp Leu Arg Glu Glu Leu Pro Gly Thr Gln Xaa Lys
            100                 105                 110

Leu Gln Arg Ser Glu Gln Ala Val Ala Gln Leu Glu Glu Glu Lys Gln
        115                 120                 125
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His Leu Leu Phe Met Xaa Gln Ile Arg Ser Trp Met Lys Thr Pro Xaa
    130                 135                 140

Leu Thr Arg Arg Arg Gly Thr Ser Pro Lys Thr His Trp Met Thr Cys
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Ser Pro Met Arg Met Ser Arg Ala Gln Pro Leu Ala Gln Glu Glu Gly
                165                 170                 175

Met Cys Leu Val Ser Met Gly Asp Thr Arg Ser Arg Pro Gly Ser Ala
            180                 185                 190

Pro Cys Thr Thr Gly Asp Pro Ile Arg Leu Thr Gly Pro Leu Arg Gly
        195                 200                 205

Ser Cys Ala Thr Leu Gln Ala Gly Thr Arg Arg Leu Glu Lys Thr Ser
    210                 215                 220

Gly His Asp His Pro Asp Val Ala Thr Met Leu Asn Ile Leu Ala Leu
225                 230                 235                 240

Val Tyr Arg Asp Gln Asn Lys Tyr Lys Glu Ala Ala His Leu Leu Asn
                245                 250                 255

Asp Ala Leu Ala Ile Arg Glu Lys Thr Leu Gly Lys Asp His Pro Ala
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Val Ala Ala Thr Leu Asn Asn Leu Ala Val Leu Tyr Ser Ala Glu
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agg aag aag gaa agt aag gac att gcc agc aaa tat ctt aca tct cat   96
Arg Lys Lys Glu Ser Lys Asp Ile Ala Ser Lys Tyr Leu Thr Ser His
            20                  25                  30

cag cct ata ctg tgt ctc ctg acc act cct aac tgc aaa gga tgc tgg   144
Gln Pro Ile Leu Cys Leu Leu Thr Thr Pro Asn Cys Lys Gly Cys Trp
        35                  40                  45

gaa aaa aag agc att gta gct ttt cca gcc tct gtg gta ggc gca gat   192
Glu Lys Lys Ser Ile Val Ala Phe Pro Ala Ser Val Val Gly Ala Asp
    50                  55                  60

aag gga tta gag ttg ggt gtt act gaa tca atg tat cag aca ctt ctc   240
Lys Gly Leu Glu Leu Gly Val Thr Glu Ser Met Tyr Gln Thr Leu Leu
65                  70                  75                  80

agt cag gct aga gcc aga ttt aac tagatttagc aggaaaagta tgtttctttc  294
Ser Gln Ala Arg Ala Arg Phe Asn
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ggaacagtag acactgggga accctagagg ggggaggttg ggagtagggg aagggttgaa 474

aaatgattgg gtactatgct cactacctgg gtgatgggat catttgtaca ccaaatgcca 534

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			20					25					30		
Gln	Pro	Ile	Leu	Cys	Leu	Leu	Thr	Thr	Pro	Asn	Cys	Lys	Gly	Cys	Trp
		35					40					45			
Glu	Lys	Lys	Ser	Ile	Val	Ala	Phe	Pro	Ala	Ser	Val	Val	Gly	Ala	Asp
	50					55					60				
Lys	Gly	Leu	Glu	Leu	Gly	Val	Thr	Glu	Ser	Met	Tyr	Gln	Thr	Leu	Leu
65					70					75					80
Ser	Gln	Ala	Arg	Ala	Arg	Phe	Asn								
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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US00/10651

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL : 800/3, 9, 14; 435/320.1, 325; 530/350, 387.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/3, 9, 14; 435/320.1, 325; 530/350, 387.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, MEDLINE, BIOSIS, EMBASE, SCISEARCH, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AUSUBEL, et al., Short Protocols in Molecular Biology. US: Wiley and sons, Inc., 1995, 3rd ed., chapter 13, pages 53-61, all pages.	1-43
Y	GUNSTER et al. Identification and characterization of interactions between the vertebrate polycomb-group protein BMI1 and human homologs of polyhomeotic. Molecular Cell Biol. April 1997, Vol. 17, No. 4, pages 2326-2335, whole document.	1-43
Y	NAYA et al. Tissue-specific regulation of the insulin gene by a novel basic helix-loop-helix transcription factor. 15 April 1995, Vol. 9, pages 1009-1019, whole document.	1-43

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

11 AUGUST 2000

Date of mailing of the international search report

29 AUG 2000

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Authorized officer

PETER PARAS

Telephone No. (703) 308-8340

Form PCT/ISA/210 (second sheet) (July 1998) *

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/10651

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ROMANOWSKI et al. XMCM7, a novel member of the Xenopus MCM family, interacts with XMCM3 and colocalizes with it throughout replication. Proc. Natl. Acad. Sci. September 1996, Vol. 93, pages 10189-10194, whole document.	1-43
Y	ZILBERMAN et al. Evolutionarily conserved promoter region containing CArG*-like elements is crucial for smooth muscle myosin heavy chain gene expression. Circ. Res. 23 March 1998, Vol. 82, No. 5, pages 566-575, whole document.	1-43

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US00/10651

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

G01N 33/00; A01K 67/00, 67/027, 67/033; C12N 15/00, 15/02, 15/09, 15/63, 15/70, 15/74; C07K 1/00, 14/00, 16/00, 17/00